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PUBLISHED QUARTERLY BY THE SOCIETY
COLUMBUS, OHIO

Entered as second class matter April 11, 1908, at the Post Office at Columbus, Ohio, under the Act of Congress of March 3, 1879.

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The Entomological Society of America

Volume VIII SEPTEMBER, 1915 Number 3

THE FORMATION OF THE MIDDLE MEMBRANE IN THE WINGS OF PLATYPHYLAX DESIGNATUS WALK.

WILLIAM S. MARSHALL,
University of Wisconsin.

There appears to be some difference of opinion regarding the terms middle membrane and "Grundmembran," their first formation and ultimate fate, and, at what stages in the development of the insect's wing they occur. Semper (10), from whose paper the word "Grundmembran" comes chose for his work the later stages of some Lepidoptera and found, between the two hypodermal layers of the developing wing, a membrane-like structure which he called the "Grundmembran"—this name has been adopted by others. Semper's view as to the origin of this membrane from "Bildungszellen" has been followed by some; we believe, however, the more widely accepted view to be that of Schäffer (9) who, speaking of the development of the wing in the Lepidoptera says: "Sehr fruh, etwas sobald die Schuppen sich anlegen, beginnt in ganz eigenartiger Weise die Verschmelzung der Flügelblatter." Again: "Est is eine von Plasmen gebildtee continuirliche Membran vorhanden die ich als 'Grundmembran' des Epithels bezeichnen will." Later the same worker says: "die Grundmembranen bei der Flügelblatter sich bereits dicht auf einander verschmolzen sind. Der Flügel erscheint so aus zwei Epithelien zusammensesetzt, von denen gegen eine in der Mitte gelengene Membran pfeilartige Fortsatze auslaufen."

What one might call the generally accepted view regarding the middle membrane is this: The basement membrane covering the hypodermis of the young developing wing is similar to that

on other parts of the body and, in the very young wing bud, the continuation of the one into that of the other can be easily seen. The young wing is formed by a fold of the hypodermis which at first remains unchanged and the two walls of the fold come to lie adjacent to each other, they finally come together and the two opposed basement membranes fuse to form the middle membrane. This happens early in the development of the wing and has been seen, clearly by some observers, indistinctly by others. Later in the development when the hypodermal cells have assumed their characteristic spindle shape this middle membrane persisting, or a new one forming, has been clearly seen as a well marked layer passing through the median portion of the wing and connected at either side to the hypodermal cells by long strands. This is the layer first described by Semper (10) and Schäffer (9) and it has been seen by nearly all those who have studied the development of an insect's wing by means of sections.

It might be well to give a few views of those who have studied the development of the wings of insects although not trying to make such a series of quotations complete. Comstock and Needham (1) working with beetles say: "It is also important to note that the basement membrane of the hypodermis of the wing differs in no respect from that of the hypodermis of the body wall, and is continuous with it. In the thinner parts of the wing the two basement membranes melt and fuse, this forming what has been termed the middle membrane of the wing."

Mercer (6) in speaking of the Lepidoptera says: "In sections of the wing buds made at this time (fifth larval stage) the so-called middle membrane is seen only with difficulty. This has given rise to the belief that it disappears at this time. Later when the wings become more opaque, i. e., in the pupa stage, the two basement membranes are again easily seen." Again the same author says: "Students of the subject have been confused by descriptions of three different structures, the basement membrane of the hypodermis, the middle membrane of the larval wing buds, and the 'Grundmembran' of the pupal wing; when in reality there is only a single structure, the basement membrane."

Mayer (5), who worked with Lepidoptera, says: "The middle membrane has disappeared as such, and in its place one

finds a delicate membrane lining the whole interior of the wing bags. This is the 'Grundmembran' of Semper." The figure to which Mayer refers for illustration of the middle membrane is taken from what he labels a mature larva; it is very similar to what we have shown in figure eight which is of an early pupa, this does not show any thing so membrane-like as Mayer has drawn and called the 'Grundmembran' in several of his later figures. Again he says: "The inner ends of these spindle-shaped cells are often seen to be fused to a double membrane (middle membrane), occupying the space between the two walls of the wing pad. In very old larvæ, however, this membrane is usually absent, and the inner portion of the cells which constitute the wing tissue end free."

Tannreuther (11), working with Lepidoptera found that "the basement membrane of the larval hypodermis is continuous over the sides of the evaginated cavity, which in later stages of development becomes the middle membrane of the larval wing. The hypodermal cells with their long fibre-like threads remain attached to the basement membrane which is continuous with that of the hypodermis." Again, in speaking of the larva in the fourth instar, he says: "The basement membrane is not so distinct, as the larva (e) at this stage differ in the sharpness of this membrane. In some individuals it is scarcely visible, in others it can only be determined by the ends of the hypodermis cells or fibres." In speaking of the prepupal wing he says: "The basement membranes of the evaginated cavity become united to form the middle membrane of the adult wing."

The following quotations are from Krüger whose work was principally with Coleoptera. "Dort wo die beiden Flügellamellen aneinanderstossen, zeigt sich schon, wie bemerkt, die erste, schwache Bildung einer Grundmembran. Sie geht offenbar aus den Zellen der Hypodermis hervor, was allerdings erst in spateren Entwicklungsstadien klar erkannt wurde. Die Grundmembran ternnt noch als deutliche Scheidewand bei die Flugellamellen."

Powell (7) in his work on the development of the wings of certain beetles, speaking of the larvæ shortly before pupation. "The basement membrane which throughout the development of the wing is very thin and not easily discernable, becomes

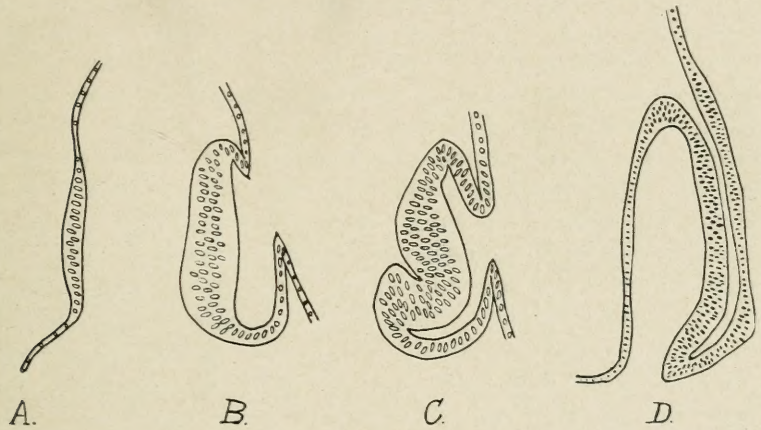
more or less degenerated during the prepupal period and in places the bases of the cells either end free or become fused and anastomosed with each other."

It might also be well to give a very brief account of the early development of the wings of *Platyphylax* taken from an earlier paper, Marshall (4).

In the earliest, newly hatched, larvæ sections through the thorax fail to show any modifications of the hypodermis at those areas where the wing rudiments will later appear. Very soon, probably in the second instar, four small, disk-like thickenings can be seen; they are formed by the elongation of a few cells of the hypodermis (Text-figure I, A) and are found on each side of the wing-bearing segments, dorsal to the legs. These disks, which remain continuous with the hypodermis, soon invaginate and each, sinking below the surface, forms a small pocket, the peripodial cavity, at the bottom of which the disk is situated (Text-figure I, B). The hypodermis consists of a single layer of cells with a basement membrane along its inner surface, this membrane is the same on the disk and on the surrounding hypodermis both of the peripodial cavity and that covering the thorax. The disks never recede far from the surface from which they invaginated and soon commence to evaginate (Text-figure I, C). The peripodial cavity remains in communication with the exterior through the peripodial pore, the opening formed by the original invagination. At first the invaginated disk was, from a surface view, circular, it gradually elongates and when evagination takes place the wing rudiment is like a flattened sack; each wall of this sack consists of a single layer of hypodermal cells and a basement membrane which forms the inner, adjacent, part of each wall. In this way the basement membrane of each wall comes to oppose and lie adjacent to that of the other wall, and, as the walls come closer and closer together, the two basement membranes lie against each other, touch, and apparently fuse. This is not true over the entire inner surface as here and there this fusion of the two layers has not occurred and certain elongated free spaces remain, these are the developing wing veins.

The wing rudiment increases in size and a slight bending is necessary in order that it may remain within the peripodial cavity; the expulsion from this cavity takes place shortly after the larva has closed its case preparatory to pupation and the

young developing wings become external (Text-figure I, D). The wing veins become clearly discernable from a surface view and in section they appear as empty spaces alternating with those parts where the two layers of the wing remain connected. While still within the last larval skin the wings become so large that, confined as they are by this covering, a second folding becomes necessary; during this period the wings attain their greatest thickness. When the last larval skin splits and the pupa is free the wings straighten and grow much thinner. The cuticular covering now formed by the wings soon becomes too small for their growth and another period of folding ensues, the wings remain thus folded until the emergence of the imago when they unfold and assume their definite form and shape.



TEXT-FIGURE I.

Four views of the young developing wing of **Platyphylax designatus.** A, thickening of the hypodermis; B, after invagination of this same thickened portion; C, the wing rudiment has evaginated, but still lies within the peripodial cavity; D, soon after the wing rudiment has left the peripodial cavity and become external. In all figures the external surface of the larva is to the right; the hypodermis only is represented, the cuticula not drawn. Figs. A, B and C, × 220; D, × 105.

After evagination of the imaginal disk each wing rudiment consists of two layers of hypodermal cells folded down into the peripodial cavity and each wall of the fold is a layer of cells similar, except in its greater thickness, to the continuous hypodermal layer forming the wall of this cavity. The two layers of the rudiment lie close to each other but in the early stages of development do not touch along any part of their opposing surfaces (Fig. 1, A). Each layer is seen in section to be com-

posed of a single row of cells with ovoid nuclei which are arranged in two or three apparent rows. The long narrow cells are so crowded together that the nuclei have lost their regular linear arrangement and have been pushed towards one end in the cell to which they belong. The nuclei are situated more in the basal half of each layer so that there is a thicker portion of protoplasm along the free surface of the hypodermis than between the nuclei and the basement membrane. In the specimens of *Platyphylax* examined the cell boundaries, which have been figured by other observers in different insects, could not be found with any great regularity and only here and there could traces of any boundaries separating the cells be seen. The hypodermis covering the thorax and that forming the peripodial membrane has a distinct basement membrane, this can also be seen on that part of the hypodermal layer forming the wing rudiment although in this last place it is not distinct (Fig.1,B.m).

The two layers of the wing rudiment, as its development proceeds, approach each other and pass through a stage in which there is but a narrow open space separating them from each other; here and there this open space is wider forming large openings, the developing wing veins. Narrow open spaces are also noticed between the cells of each layer, these do not as yet entirely separate the cells but appear only in the basal region; this is, however, the beginning of that separation of the cells which finally results, with the migration of the nucleus and protoplasm to one end, in the formation of the elongated, spindle-like cells which have been described in the developing wings of a number of insects. An endeavor to find a basement membrane in the wing rudiment shows that it is not distinct and continuous, it can sometimes be seen but cannot be traced for any considerable distance along the basal surface of either layer. Other workers have observed the basement membrane at this stage in different insects although, as quoted in the introductory remarks, all have not seen it with equal distinctness.

As the development of the wing goes on its two layers finally approach each other and their inner surfaces touch except where the developing wing veins are present. The two basement membranes should now lie adjacent to each other and fuse to form the middle membrane. An examination of sections at this stage shows however, that a continuous median membrane separating the two layers of the wing from each other cannot

with certainty be found. One does however find, running through the middle of each section, a continuous, narrow, lighter zone which is connected with and separates the two layers of hypodermis from each other (Fig. 3). This zone is without definite boundaries, and, from the darker protoplasm of the adjacent hypodermal layers, there are numerous small processes extending into it so that it is impossible to trace any definite line which might separate the parts from each other. Another change one notices at this stage is in the gradual moving of the nuclei of each layer towards the outer surfaces of the wing; most of these nuclei are no longer, as formerly, grouped in the basal half of each layer but are now fairly well scattered in all its parts. This is well shown by comparing figures one and three.

It is apparent that this light median zone remains for but a short time during the development of the wing. Slides through wings a little older than the last described fail to show any median zone which can be recognized as lighter in shade than the rest of the wing, but, in the same median position, one can still see the same zone but it is now darker than the rest; the sections showing this were prepared and stained in the same way as the slides showing the lighter median zone. The two layers of the hypodermis no longer lie close against this median zone but have moved slightly away from it, not leaving a clear entirely open space, but each layer of the hypodermis is connected with the median zone by numerous protoplasmic strands separated from each other by vacuoles which are irregular in outline. This makes each side of the median zone much lighter and this contract coupled with a probable increase in the density of the protoplasm of the middle zone, might account for its now appearing darker than the other parts of the slide (Fig. 4). There are now, excepting the cuticular covering, five layers shown in each section of the wing: 1, two outer layers, the original hypodermis, which have decreased in width and now form but a part of the entire section; 2, along the inner surface of each of these is a lighter layer composed of numerous vacuoles separated from each other by protoplasmic strands which connect the two outer layers, 1, with 3, a median layer which appears slightly darker than the other parts of the section. None of these layers has a distinct boundary. This last median layer is present in the developing wing of *Platyphylax* and forms what is commonly known as the middle membrane; this,

as will be shown, disappears and is replaced by a second one which is Semper's "Grundmembran." The accepted name, middle membrane, is so well established that the introduction of a new term, such as middle lamella or middle layer, would be futile; the old term will be adopted both for the earlier and the later layer although we cannot see in *Platyphylax* that there is a true membrane present in the median part of the developing wing.

In many sections at this age, and later, there can be found running through this middle layer small darker dots and short lines which would correspond to the middle membrane of other observers (Fig. 5). These are more or less distinct in different sections but do not show continuously for any great distance in any section and we are unable to find any regular structure strictly homologous to a membrane. The strands of protoplasm which connect this middle layer to the outer layers will be spoken of as the perpendicular strands.

All the stages so far described can be found in the developing wing while it is still within the peripodial cavity, in fact many internal rudiments show stages more advanced than these. We also find in these stages as well as later ones a number of dividing nuclei, the mitotic figures were nearly all found near the outer surface of the developing wing and away from that portion where the nuclei are most crowded together.

The middle membrane can be recognized when the perpendicular strands and the vacuoles between them first become clearly differentiated as layers of the wing. With the growth of the wing changes take place in the relative thickness of the different layers, the two original hypodermal layers decrease in thickness and the layers just inside of them, composed of the perpendicular strands and vacuoles, increase in thickness to finally, as will be seen later, occupy by far the largest part of the wing. The perpendicular strands are not straight but branch and divide and are generally curved for part of their length, such irregularities are more noticeable in the older stages when the strands are longer. Each strand appears to pass from the middle membrane to a nucleus in the hypodermal layer (Fig. 6). In sections such a connection is not always discernable but no doubt holds true for a great majority of the strands. Mayer (5) says: "Each of the hypodermis cells gives rise to one, and only

one, of these processes"; and later, "occasionally a hypodermis cell is seen without any such process."

At first the increase in width of the clearer layers, that is the elongation of the perpendicular strands and the clear spaces between them, causes no appreciable changes except in the relative width of the different layers. In certain slides one can see that, scattered rather irregularly in the middle membrane, there are a number of small ovoid bodies not well stained but clearly marked off from the surrounding protoplasm. At first it was difficult to understand just what these small bodies were but from a study of different stages of development it became apparent that they were nuclei of the hypodermal layers which had wandered into the middle membrane. In wings of about the age we are now considering, (Fig. 6), one can see that many of the nuclei of the hypodermis lie along its inner margin, some are noticed protruding into the adjacent clear layer and a few are seen on the perpendicular strands. Those nuclei occupying the two first mentioned positions and some of those on the strands are similar in size and structure to the normal nuclei of the hypodermal layer, some of those on the strands however are seen to be much smaller and lighter stained than normal but are still easily distinguished by their rather distinct boundaries which mark them sharply off from the surrounding cytoplasm (Fig. 7). This enlarged view will show more clearly what happens during this wandering and that many of the hypodermal nuclei pass from their original position to the middle membrane going from one layer to the other along the perpendicular strands. During this change in their position they lose their characteristic nuclear appearance and become much reduced in size. In this last figure (7) one sees, to the right, the inner ends of a few normal nuclei (entire nuclei not drawn) that are still within but at the inner edge of the hypodermal layer, adjacent to these are two entire nuclei that have started to move towards the middle membrane; the latter nuclei are as yet normal in appearance and size. Along some of the perpendicular strands can be seen other nuclei that have already decreased in size and lost their nuclear characteristics in a breaking up and disappearance of their reticulum and in their failure to stain. Finally, in the middle membrane, can be seen many of the nuclei which have wandered from the hypodermal layers, these have entirely lost their nuclear appearance but can be distinguished by the

rather definite boundary with which each is surrounded; they persist as small, fairly regular ovoid bodies within the middle membrane, remaining visible until a considerably later stage, continually decreasing in size to finally disappear.

Comstock and Needham (1) give a different origin for the nuclei in the middle membrane. They say: "In later stages, when, after the expansion of the wing, it (basement membrane) contains distinct nuclei, there is evidence that some of these at least are derived from the hypoderm cells whose nuclei once crowded up to this level, have remained stranded here after the expansion of the wing." Later in the same work they say: "When through excessive crowding, some of the innermost nuclei have come into contact with the basement membrane at the subsequent expansion of the wing, these, seem instead to remain where they are, and to attract to themselves the slender prolongations of the neighboring cells."

In the stages which have already been described (Figs. 1 to 7), the wing, except in the earliest stage, (Fig. 1), remains of about the same thickness and any changes taking place during the formation of the middle membrane and of the perpendicular strands go on during a partial rearrangement of the contents of the wing and an increase of its area. Before the larva closes its case preparatory to pupation the wing has grown down against the base of the leg and subsequent growth in a ventral direction is checked; covered, externally, by the cuticular layer the wing is confined within a limited area which it finally fills, it then starts to fold and this is noticed in both small and large folds along the surface (Fig. 8), giving it a fluted appearance, Verson (13). During this period and also after the larval case has been closed a surface view will show another system of very much larger folds; these start at the anterior margin of the wing and finally extend entirely across it, at first there are but one or two but an increase in their number soon occurs and gives to the wing a complicated folded appearance Marshall (4, Fig. 23). This is now that stage in the development of the wing when it has reached its maximum width and the perpendicular strands their greatest length.

Sections through the wing at this period of its greatest thickness show that there has been a considerable change in its internal structure. The outer layers which have been very distinct since the beginning of the formation of the clear layers

have nearly disappeared and are now seen restricted to a narrow layer along the surface of the wing just under the cuticula. It is seen from this section (Fig. 8) that the earlier clear layers now form nearly all of the wing, the long clear spaces still separating the perpendicular strands which extend nearly to the cuticula. The middle membrane is much narrower than in the last stage but occupies the same median position and still shows a number of the nuclei which have wandered into it.

During the growth of the wing in the latter part of larval life and while the insect is in a period in which the wings would be of about the ages found in figures six and eight the middle membrane shows, in some specimens, traces of what might be taken for the remains of a membrane. Running through the center of the middle membrane there can often be seen small dark dots and rods which may in some specimens be so numerous as to show a more or less linear arrangement (Fig. 8), this is only continuous for a short distance. Most of the slides examined did not show this central linear arrangement of dark dots and rods and one could see only a few darkened dots in its place; in most of the sections examined nothing of the kind could be found. We do not believe that this corresponds to a membrane although it occupies exactly the position in which the basement membrane would be found if present.

The meaning of the wandering of some of the nuclei from the hypodermal layers to the middle membrane is not clear. As will be shown later these nuclei finally disappear and there is no apparent reason why they should leave those parts of the wing where the other nuclei are found and wander to a portion of the wing in which it is impossible to see that they are of any use. The crowding of the nuclei due to the increase in width and folding of the wing might necessitate a decrease in their number but at this stage there still remains a thin outer layer along the surface of the wing which is nearly free from nuclei (Fig. 6) and into which other nuclei might be pushed. That an accretion to the mass of the middle membrane is needed and supplied in this way is not possible as the middle membrane soon after the nuclei have wandered into it, begins to decrease in thickness and to ultimately disappear. During the stages already described dividing nuclei can often be seen within the hypodermal layer so that nuclei are both being formed in this layer and also lost to it from this change in their position.

The wings, after the last larval skin has finally been cast, remain for a short time in their folded condition; they then unfold, decrease in thickness with an increase in their area. As a result of this the old hypodermal layers, just under the cuticula, are spread as a continuous layer in this position and have apparently received a considerable amount of the cytoplasm which was formerly in the perpendicular strands when these became shorter and thinner (Fig. 9). The nuclei, which in the last stage were all in the perpendicular strands, have nearly all wandered into the layers under the cuticula; a few still remain in the strands from which they later disappear. The middle membrane has become much thinner and, instead of being a fairly continuous layer, it here and there now assumes a zigzag shape; along its course can be seen very small and somewhat ovoid bodies, these are all that remain of those nuclei which, at an earlier stage, wandered into this layer from the hypodermis.

During that period in the life of the pupa in which its body contracts and shortens the changes, noted in the last paragraph, are continued and become more marked. The nuclei of the perpendicular strands have all passed from these into the old, outer, hypodermal layers in which they now are arranged in a fairly even layer. The protoplasm does not in these layers become even but in many places surrounds each nucleus in a triangular mass, these at the base are connected with each other but the apex of each points towards what remains of the middle membrane and is, in most cases, extended out into one of the perpendicular strands (Fig. 10). The perpendicular strands have become thinner and most of the protoplasm that they contained has entered the outer layers of the wing. The middle membrane no longer extends as a continuous layer through the median part of the wing but its zigzag course becomes more marked until finally it separates into a number of strands and can no longer be followed continuously as in all the earlier stages. This disappearance of the middle membrane becomes more marked until all traces of it are lost, the perpendicular strands then either pass across the wing from one surface to the other or they end blindly at some place along such a course. The failure to see all of the strands connected with both layers of the hypodermis is undoubtedly in part due to a study of thin sections. Many of the nuclei which earlier wandered into the middle membrane from the hypodermal layers

are still present but now restricted to the perpendicular strands (Fig. 11).

The wings reach their maximum thickness after the contraction of the pupa and they then lie stretched over and at the sides of its body; this chitinous case which now encloses them while ample in extent for the wings in their present condition is not sufficient to allow of the next growth, that of an increase in surface area. When this occurs it is necessary for the wings to decrease in thickness and become very much folded which folding continues until the imago is ready to issue from the pupal skin. To the perpendicular strands that pass across the wing from one surface to the other Mayer (5) has assigned a probable contractile power and to this attributed the drawing together of the two surfaces of the wing. This would account for the decrease in thickness and the limited area of the cuticular sack which now encloses the wings would necessitate the folding which becomes so marked during the remainder of pupal life. The narrowing of the wing is noticeable before its folding has commenced.

During the process described above certain changes take place in the different layers of the wing, of these the most noticeable is the reforming of the middle membrane which again occupies its old place in the median part of the wing (Fig. 12) and is now thinner and more membrane like than during the earlier stages before its disappearance. The perpendicular strands again pass from the hypodermis to the middle membrane and are irregular and branched. The degenerated nuclei which earlier wandered from the hypodermal layers to the middle membrane and which, upon the disappearance of the latter, remained on the perpendicular strands (Fig. 11) are again found in the middle membrane. In the hypodermal layers many nuclei are seen which have wandered away from the outer surfaces of the wing and come to lie between the hypodermis and the middle membrane. These are the nuclei of those cells, trichogens, from which later will develop the hairs upon the surface of the wings. It is at once noticed that these trichogens are more abundant upon one side of the sections than upon the other; this fact, knowing that there are many more hairs upon the dorsal than upon the ventral surface of the wing, enables one to distinguish these surfaces from each other rather early in pupal life.

The wing, as this last folding continues, decreases more and more in thickness (Fig. 13) but structurally there is no noticeable change. Here and there places are seen in the sections where the hypodermis and middle membrane have increased in thickness (Fig. 14) but such places are apt to be near the margin of the wing. The cause of this is not known unless it can be due to the folding of the wing which may push the hypodermis and the middle membrane in such a way as to increase, at certain places, the thickness of each.

After the folding of the wing has reached its maximum (Fig. 15) certain changes have taken place. Most noticeable of these is the final disappearance of the middle membrane and of the small degenerated nuclei which it contained. After this has occurred the perpendicular strands again pass entirely across the wing and directly connect the two hypodermal layers with each other. These layers are now thinner and their nuclei are so arranged that the longitudinal axis of each lies parallel to the surface of the wing.

No marked change is noticeable in the wing after the adult insect has emerged (Figs. 16 and 17). The wing has become a little thinner and the hypodermal layers show a decrease in amount and their nuclei are smaller. The activities of the different layers have ended and there is little left within the wing of what was present during the early stages of its development and growth.

From the foregoing account it can be seen that in *Platyphylax* the term middle membrane cannot be used to designate a true membrane but rather as the name for the thin layer of protoplasm occupying a median position within the wing. As has been noted by others this layer is not continuous during the entire development and growth of the wing but disappears and is reformed in the same place. Of these two structures the latter is the more membrane like.

In the preparation of the material two or three of the commoner sublimate, acetic acid fixitives were used and the slides stained with Delafield's hæmatoxylin or with alum carmine.

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EXPLANATION OF PLATES XX–XXII.

All figures drawn with a camera lucida.

B. M., basement membrane.
Cu., cuticula.
Hyp., hypodermis.
M. m., middle membrane.
Per. cav., peripodial cavity.
Per. mb., peripodial membrane.
Pp. s., perpendicular strands.
Tr., trichogens.

Figures eight to seventeen inclusive have been drawn with the same magnification to allow an easy comparison of the relative thickness of the wing at these different stages of development.

Plate XX.

Fig. 1. Section through one of the two layers of hypodermis that form the internal wing rudiment. This figure shows the position of the crowded nuclei as more in the basal part of the layer. ×875.

Fig. 1A. Transverse section of the entire wing rudiment from which the preceding figure was taken. × 105.

Fig. 2. The middle part only of a section through an internal wing rudiment. The outer part of each layer of the hypodermis is not drawn. The two layers have nearly come together and only a slight open space can be seen between them. The separation of the cells along their sides has started and a few of the narrow spaces between them can be seen. × 1100.

Fig. 3. Section through a wing rudiment after the two layers have come together and a narrow, lighter zone has appeared between them. × 875.

Fig. 3A. Transverse section of entire wing rudiment from which preceding figure was drawn. × 105.

Fig. 4. Median part only of a section through a wing rudiment. This shows the early formation of the middle membrane and of the perpendicular strands. × 1100.

Fig. 5. Section through a young external wing, lateral stage than preceding figure. × 1100.

PLATE XXI.

Fig. 6. Section of a wing at a later stage showing the wandering of many of the nuclei to the middle membrane in which a number of these nuclei, much reduced in size and clearness, can be seen; other nuclei are visible on the perpendicular strands. To the right a small outer portion of the hypodermis and the cuticula have not been drawn. × 1100.

Fig. 7. A small part of the same section more highly magnified to show the wandering of the nuclei from the hypodermis to the middle membrane. Only the inner edge of one layer of hypodermis is drawn and, in it, the ends of five nuclei. Opposite this is shown a part of the middle membrane and between these two the perpendicular strands on which are seen some of the wandering nuclei. The position of this view is shown by the space between the two lines at **a**, in the preceding figure. × 1700.

Fig. 8. Section of a wing at a later stage, shortly before the casting of the last larval skin. All the nuclei in the middle membrane are reduced in size and the perpendicular strands greatly elongated. × 740.

Fig. 9. Section of a wing at a still later stage of development. The middle membrane in which are seen a few of the nuclei that have wandered into it from the hypodermis is narrow and much more membrane like than in the preceding figures. × 740.

PLATE XXII.

Fig. 10. Section of a wing from a pupa after the last larval skin has been cast, the body contracted and the wings straightened. The nuclei of the hypodermis are nearly all arranged in a single row along the surface and many of the perpendicular strands extend entirely across the wing. The middle membrane is no longer continuous. × 740.

Fig. 11. A little later stage showing that at this part of the section the middle membrane has entirely disappeared. Cuticula not drawn. × 740.

Fig. 12. Section from the wing of a pupa before its final folding has commenced. The middle membrane has again formed as a continuous layer. × 740.

Fig. 13. Section of a pupal wing after the last folding has started. Cuticula not drawn. × 740.

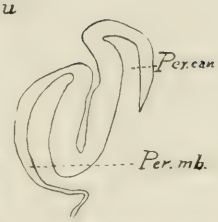
Fig. 14. Section of another wing of about the same age. Cuticula not drawn. × 740.

Fig. 15. Section through the wing of an old pupa. The wing of this specimen is much folded and shows the entire and final disappearance of the middle membrane. The perpendicular strands again pass across the wing. Cuticula not drawn. × 740.

Fig. 16. Section of the wing of an imago shortly after its emergence. The old hypodermis is now represented by a thin layer of protoplasm containing a few shrunken nuclei. × 740.

Fig. 17. Section through the wing of an adult. × 740.

Fig. 1



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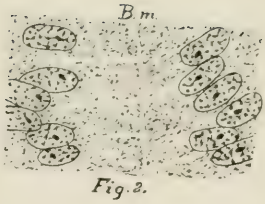

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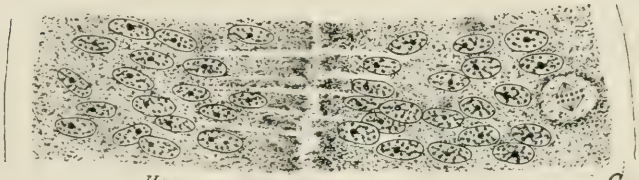

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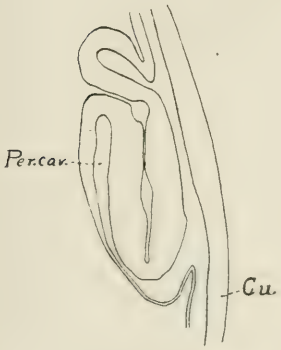

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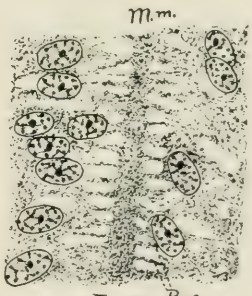

Fig. 4

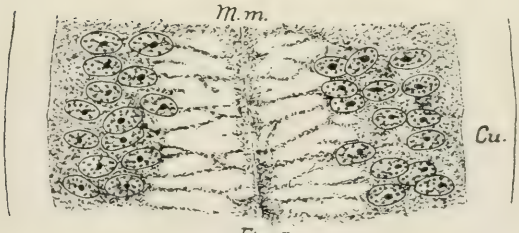

Fig. 5.

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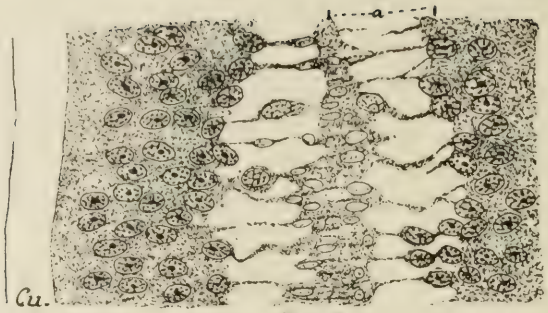

Fig. 6.

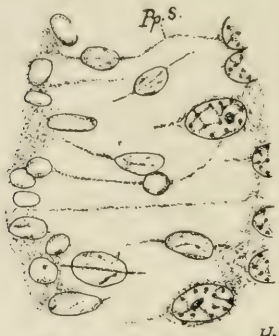

Fig 7.

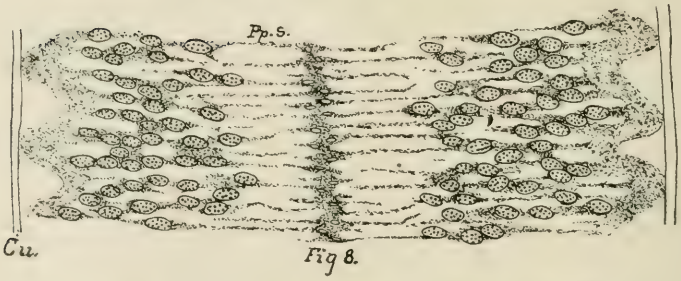

Fig 8.

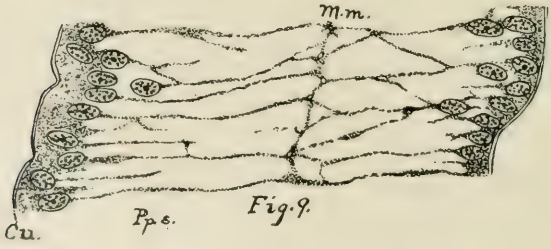

Fig. 9.

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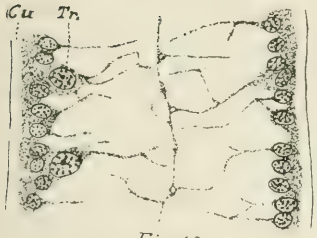
Fig. 10.

Fig. 11



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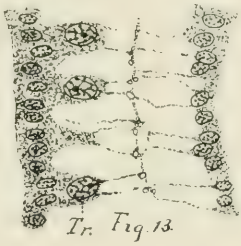

Fig. 13.

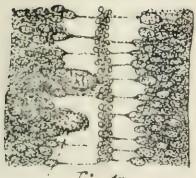

Fig. 14

Fig. 15.

Fig. 16.

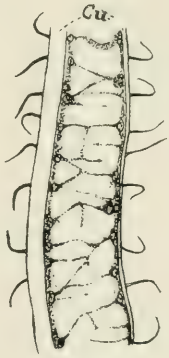

Fig. 17.

W. S. Marshall.

BEHAVIOR OF ANOPHELES ALBIMANUS WIEDE. AND TARSIMACULATA GOELDI.*

JAMES ZETEK,
Entomologist, Republic of Panama.

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INTRODUCTION.

This paper is largely a report of definitely observed and demonstrated flights of *Anopheles albimanus* Wiede. and its racial variety *tarsimaculata* Goeldi. Darling (1912) has shown that these two species are the most important ones in the transmission of malaria on the Canal Zone. *Albimanus* is by far the commonest Anophelene about settlements, but at times, and locally, the race *tarsimaculata* appears in large numbers. At the salt-marsh northwest of Gatun, breeding of these two forms was so vigorous (apparently demoralized) that unlimited variations in the middle white band on the palpus were noted, this band being often represented by only a few white scales. For all practical purposes, however, these two forms may be considered as a single species.

PRACTICAL VALUE.

The answer to the question, aside from a purely scientific importance, "what is the value of the knowledge of the behavior of mosquitos?", is that through this knowledge we are better

*This paper was presented by title at the 1913 meeting, accompanied by a blue-print which gave the essential data concerning the flight studies. While in the interior of Panama, the writer hoped to revise the manuscript, but the mule which carried the packs containing the paper met with an accident while fording a stream and the manuscript was badly spoiled. It had to be rewritten upon the writer's return, from the original notes preserved in his laboratory. Hence this extreme delay.—Z.

able to direct our crusades of extermination against these common enemies of mankind. To stop the spread of insect-born diseases is to save lives, dollars and to increase comfort.

Environment and organism are so closely knitted together that a change in the former causes a change in the physiological state of the organism. And if the animal cannot endure this change in its abode, it must seek a new, more suitable home, or perish. Thus our knowledge of the behavior of mosquitos gives us clues toward the production of just such changes in the environment of the mosquitos which they cannot withstand. Hence human hand may, by means of relatively simple measures, reduce the population of disease-spreading species far below the critical point of danger, if not actually wipe them out.

But to actually wipe out of existence in a given region all such malefactors would probably be prohibitive on account of its cost. Constant vigilance and effort are required to maintain a humid, tropical area free from malaria or yellow-fever. The conversion of Colon and Panama cities from filth and disease-propagating centers into unusually healthy places, stands out boldly as a proof of what can be done. It means new birth to business opportunities and makes life worth something. The knowledge of the behavior of insects has, therefore, undisputed practical value.

METHODS OF STUDY.

1. *Field Study.* This is by far the most productive means of getting at the correct interpretation of the dynamic relations of the mosquitos in their normal environment.
   - A. By Direct Observation:
     - i. From a boat, at all times of the day and night.
     - ii. Observations on land, at the breeding place and townsite, with and without lights, in tents, with domestic animals, etc.
     - iii. Collection and examination of adult mosquitos.
   - B. By Experimental Data:
     - i. Adult mosquitos were attracted into large mosquito-bar nets, at the breeding place, by West Indian negroes. The mosquitos thus caught were sprayed with an aqueous anilin dye (Zetek 1913-a) and liberated at dusk at the same place where sprayed. The mosquitos caught at the townsite and elsewhere were tested for the presence of color.
     - ii. By means of intercepting planes, the sides of which were coated with transparent tanglefoot.
2. *Laboratory Study.* Due to the separation of the mosquito from its normal environment, but few laboratory experiments were attempted. These were simple and only to learn the responses to single factors, such as light intensity.

ACKNOWLEDGMENTS.

The author is particularly indebted to Surgeon-General, Wm. Crawford Gorgas, formerly Chief Health Officer of the Panama Canal Zone; it is due to his efforts that entomological study on the Isthmus at this time was at all possible. To Mr. Joseph A. Le Prince, formerly Chief Sanitary Inspector, acknowledgments are due for his support and constant encouragements throughout the work.

The author is indebted to the following gentlemen for assistance given: Mr. James B. Shropshire, detailed to assist him; Dr. A. J. Orenstein, former Assistant Chief Sanitary Inspector; Dr. Samuel T. Darling, former Chief of Laboratory, Ancon Hospital; Mr. J. A. Corrigan, Sanitary Inspector at Gatun; Dr. E. Garcon of Gatun Dispensary; Sanitary Inspectors Messrs. C. B. Chinn, Geo. Parker, E. F. Quinby, C. H. Bath, Chas. P. Crafts, A. R. Proctor and S. P. Verner. The writer acknowledges with gratitude the helpful criticisms and encouragements of Dr. L. O. Howard, Chief of the Bureau of Entomology, U. S. Dept. Agric., and of Messrs. August Busck and Frederic Knab of the same bureau. Only those intimately acquainted with mosquitos of tropical America, and who have worked under the enervating strain of its moist, hot climate, can appreciate the services rendered by these gentlemen.

REGIONAL ORIENTATION.

The Panama Canal Zone is a typical sample of the humid, torrid zone, characterized by an uniformly even climate, with very little seasonal change, with a wet and a dry season, a fairly heavy rainfall, high humidity, prevailing north and northwest winds, and a prolific and luxuriant biota. Of mosquitos alone it yielded about 130 species. For a good account of the Zone and the problems of mosquito control, see Jennings 1912, pp. 131-141.

The town of Gatun is located seven miles from the Atlantic entrance, and is the site of three flights of locks which, in less than a mile, raises the level of water from sea-level to 85 feet in Gatun lake. To sustain this level of 85 feet in the lake, a number of dams had to be built, the largest of them being the famous Gatun Dam, nearly 1.5 miles long and 105 feet high.

About 0.75 mile west of Gatun, this dam is cut through by a large spillway. Here was located a small camp (Spillway Camp), consisting of several labor barracks, a dispensary and a hotel. A mile north of this place, and nearly a mile northwest of Gatun, is a low, flat land, the highest portion of which is a small dome 20 feet high. The major portion of this region is below the 10 foot contour, most of it a salt-marsh. This region marks the early workings of the old French Canal Company, and is bordered to the east by a deep channel known as the Old French Canal. The excavations of this pre-American attempt were dumped along the banks of this flat land. Thus perfect drainage was made impossible; however, the region never gave serious trouble to the sanitary inspector, until the latter part of 1912 and the beginning of 1913. At this period countless, in fact unbelievable, numbers of *Anopheles albimanus* Wiede., its racial variety *tarsimaculata* Goeldi and *Aedes tæniorhynchus* Wiede., invaded the towns of Gatun and New Gatun, and it was soon evident that they were breeding in this salt-marsh. It is this area that gave such good opportunities to demonstrate mosquito flight.

East of this marsh is a low island, partly covered with a dense vegetation, and the rest of it composed of barren hydraulic fill. No serious breeding occurred here, however, shade from the hot sun was present for the mosquitos traversing the island. This was the main site for the LePrince experiment recorded in this paper.

South of the island is a small neck of land, mostly above the ten foot contour, which is covered with luxuriant vegetation. This tangle of growth was cut down and burned in February, 1913, and the measure greatly reduced the number of mosquitos for the time being.

From the breeding place to Gatun is a rise of about ninety feet, but east of the locks the land ranges in height from fifty to a hundred and fifty feet, with isolated knolls of 110 to 160 feet. It is unusually well-drained and oiled, and it can be said that no *Anopheles* breeds within this treated area—a great credit to the sanitary inspector in charge, J. A. Corrigan.

The population numbered at the time about 4,500, distributed as follows: 800 white Americans, 1000 West Indian negroes, 200 East Indians, 1500 Spaniards and 1000 all others.

The homes of the Americans are well built, well screened with 18-mesh copper screen, and are raised on concrete posts. Weekly inspections are made of screening and floors and defects, such as holes or cracks through which mosquitos can enter, are quickly repaired. A daily search for mosquitos is made in all barracks by expert negroes and thus many *Anopheles* are killed before they become dangerous. All doors have self-closing devices.

The quarters of the negro, Spaniard and East Indian are likewise well screened and elevated from the ground. The difference between them and those of the Americans is that more people sleep in labor barracks, and due to the increased perspiration, as well as less body cleanliness, these barracks become veritable traps for mosquitos. Advantage was made of this fact and the "C. H. Bath" mosquito traps were attached to such buildings and through them large numbers of mosquitos were caught.

At New Gatun, a native village adjacent to Gatun proper, the conditions are much the reverse. The dominant figure here is the West Indian, and his home, devoid of screening, rivals in capacity our New York tenements. The population was about six thousand. The only effective anti-malarial measures are free medical aid, good ditches and the ceaseless dripping of larvacides. Orenstein (1912-b) reports three times as much malaria originating in New Gatun as in Gatun proper, and this increase is due to exposures to infection through lack of screening.

In daily habits the people vary greatly. When the day's work is ended, the American usually seeks his only place of amusement—the Y. M. C. A., or he remains in his room. In either case he is fairly well protected from Anophelenes. Saturday nights and Sundays, since the Canal Zone is "dry" territory, he procures his alcoholic preference in Colon or Panama cities, i. e., unless he keeps it in stock in his trunk.

The Spaniard delights to lounge outdoors, remaining so till late at night. Mosquitos find no difficulty in reaching them. The negro likewise prefers to roam about, and this unrest is probably but the natural reaction after a day of hard work. The practice, though, is dangerous. The negro cannot speak through a screen door; he must open it wide, and of course

must let mosquitos enter until he quits talking. The finding of a greater number of Anophelenes in negro camps is explained by this fact. At Mira Flores the writer found that a negro camp with two doors had almost twice as many mosquitos as did a similar camp with but one door, not-with-standing that the camp with two doors was farthest away from the breeding place.

The time of activity of the men is largely during the day-time, though sometimes forces have worked at night time as well. Men begin to emerge from their barracks as the first rays of the sun greet the new day, and they remain active until the last ray has departed. Mosquito activity is most pronounced during day-break and dusk. This coincidence bears a direct relation to the malarial rate.

THE ENVIRONMENT.

A. ITS COMPOSITION.

In nature the composition and dynamics of the environment are inseparable, but for convenience in the presentation of the subject, this division is necessary.

I. Physical Factors.

A. The Wind. For much of the meteorlogical data the writer is indebted to Mr. Wilson and his staff of the Weather Bureau of the Isthmian Canal Commission.

During the months of January, February and March, 1913, the prevailing winds at Gatun were from the north. A summary of the wind movement for January and March is given in table "A". Of the 744 hourly periods in January, 495 showed north winds, and 164 showed northwest winds—a percentage of 66.5 and 22.2 respectively of the total. Many winds reported as "west" or "northeast" were such by the mere addition of a dot or two on the record made by the automatic wind gauge. There were more westerly winds than northeasterly. In the month of March, northeast winds were wholly absent. In other words, the winds are predominantly from the north and northwest. This general prevalence in direction holds true for the entire year in the Atlantic section.

A. Monthly Wind Movement, Gatun, C. Z.

1. Direction, Hourly Periods.

1913	North		Northwest		West		North-east		East		Calms		Total
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Jan.....	495	66.5	164	22.2	57	7.6	25	3.4	1	.01	2	.02	744
Mar....	540	72.6	178	23.9	26	3.5	.....	.....	...	.....	...	.....	744

2. Miles per Hourly Periods.

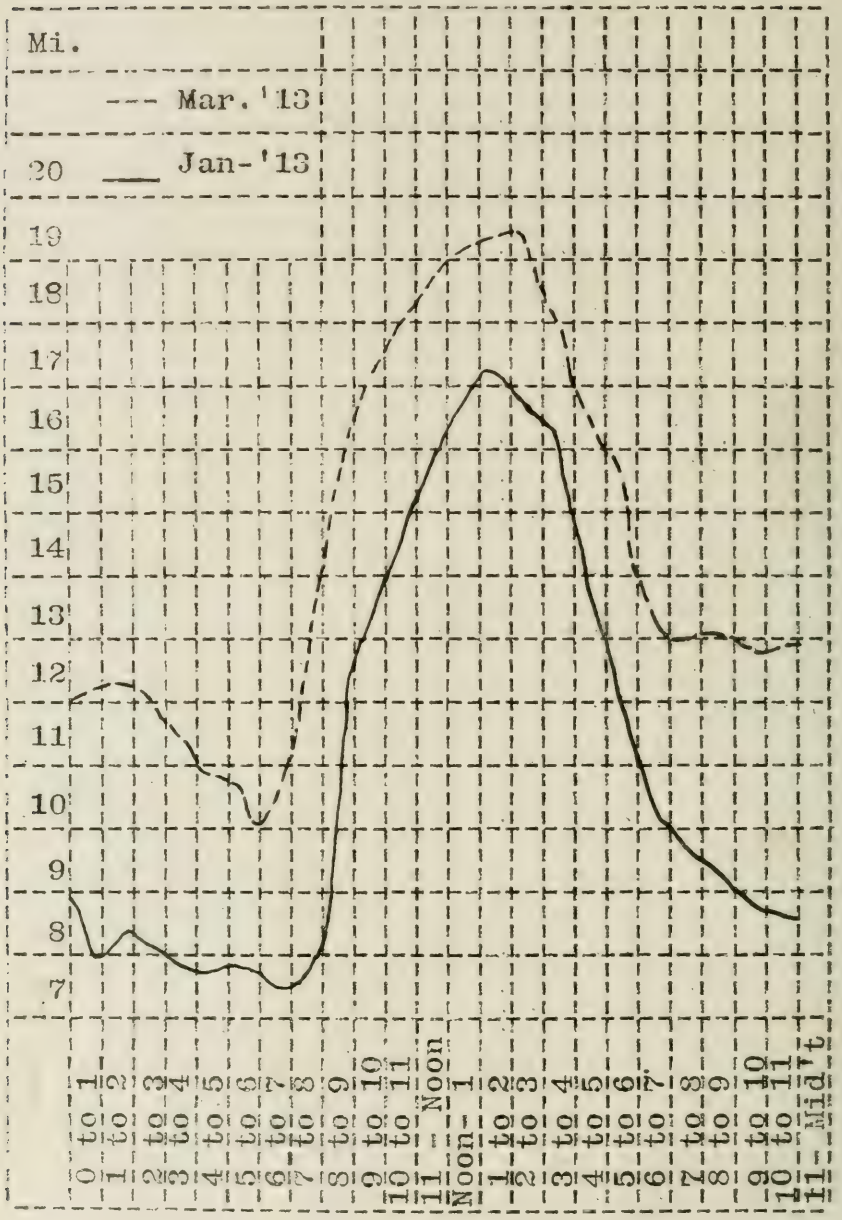
Jan.....	6379	68.0	1306	16.9	319	3.9	170	2.1	1	.....	...	.....	8175
Mar....	8655	80.7	1856	17.3	217	2.0	.....	.....	...	.....	...	.....	10728

These first three months of the year are within the dry season. This period begins the latter part of December and extends well on into April, though some years it may appear sooner or be much delayed. It is not rainless as the name would imply, for at least every third day some rain falls. But as this dry season advances, the trade winds increase in velocity and become more confined to the north. In March, for instance, there are ten percent more north winds than there were in January; in mileage this was an increase of over eleven percent.

During March, '14, the writer had been in Boquete and David, Chiriqui Province, near Costa Rica. The north winds were so dominant here and of such high velocity that the trees on the plateau leading to Boquete are limbless on the windward side, and their trunks are bent to leeward. Riding on horseback against this fierce wind, along the llanos of Santa Cruz and Dolega, was an experience which cannot be forgotten. Everywhere, everything told of its struggle to remain, even though so powerful a wind aimed at its destruction—presenting a field of intense interest to the ecologist.

A significant feature of these winds is their daily hourly range. The mean wind velocity of the months of January and March is plotted on curve, chart "B". Excepting for differences in velocity, the two curves agree. The crest is at midday. The winds show a steady diminution in velocity from about midnight till about 7:00 a. m., and a like, but more decided decrease from about 2:00 p. m. till about 9:00 p. m.

B. MEAN WIND VELOCITY. GATUN



The winds at mid-day are hot, strong and steady. Those at day-break and dusk are much milder, cooler and often inclined to be puffy.

Two charts, "C" and "D" are presented to show the characteristic of the winds at the hours when the mosquitos are most active. These charts give the mileage and direction of winds at Gatun for the hours of four to seven a. m., and five to eight p. m., during the months of Jan., Feb., and March, 1913.

CHART C. WINDS, 4–5, 5–6 AND 6–7 A. M.

Mo.	Days of Rec.		North a	North b	North c	Northwest a	Northwest b	Northwest c	West a	West b	West c	North-east a	North-east b	North-east c	East a	East b	East c	Calm	Total	Av.
		Days	16	22	21	10	6	6	2	2	3	2	1	1				1	237	7.7
Jan.	31	Miles	150	200	184	72	26	29	9	5	10	6	3	2	..	..	..	..	234	7.5
		Avr.	9	9	9	7.2	4.5	4.7	4.5	2.5	3.3	3	3	2	..	..	..	..	225	7.3
		Days	14	13	15	10	10	9	1	2	2	..	..	..	1	..	..	..	180	7.0
Feb.	26	Miles	121	115	120	51	53	39	2	6	4	..	..	..	6	..	..	..	174	6.7
		Avr.	9	9	8	5.1	5.3	4.3	2	3	2	..	..	..	6	..	..	..	163	6.3
		Days	23	22	23	8	9	8	..	..	..	..	..	..	..	..	..	..	345	11.1
Mar.	31	Miles	288	270	265	57	67	52	..	..	..	..	..	..	..	..	..	..	337	10.9
		Avr.	12.5	12	11.5	7	7	6.6	..	..	..	..	..	..	..	..	..	..	317	10.2

CHART D. WINDS, 5–6, 6–7 AND 7–8 P. M.

Mo.	Days of Rec.		North a	North b	North c	Northwest a	Northwest b	Northwest c	West a	West b	West c	North-east a	North-east b	North-east c	East a	East b	East c	Calm	Total	Av.
		Days	20	19	18	7	9	10	3	3	3	1	..	..	None			0	390	12.6
Jan.	31	Miles	290	232	203	67	79	87	23	25	20	10	..	..	"			"	336	10.9
		Avr.	14.5	12.2	11.3	9	9	8.7	8	8	7	10	..	..	"			"	310	10.0
		Days	19	19	18	6	6	7	1	1	1	N	on	e	None			0	326	12.7
Feb.	26	Miles	274	238	198	43	41	44	9	4	5		"		"			"	283	10.9
		Avr.	14.4	12.5	10	7	7	6	9	4	5		"		"			"	247	9.5
		Days	20	19	18	9	10	11	2	2	2	N	on	e	None			0	496	16.0
Mar.	31	Miles	357	305	282	117	107	114	22	15	13		"		"			"	427	13.8
		Avr.	17.8	16.6	15.6	13	10.7	10.4	11	25	6.5		"		"			"	409	13.2

During the entire three months, of the 264 hourly periods in the morning, 196 were with north winds, 76 with northwest winds, and only 19 for all others. Of the same number of evening periods, 170 were with north winds, 72 with northwest and but 22 for all others. In mileage, the north winds were stronger than those of the northwest.

B. Temperature. The Isthmus of Panama lies very near to the thermal equator. Its temperature is fairly even the whole year, ranging from about 95° F to 65° F, the general mean about 80° F. The greatest daily range of the Gatun section is 10-15°, and this is about 50% of that of the Central and Pacific sections of the Zone. As the dry season approaches, the maximum and minimum (absolute) temperatures fall gradually, on the average 1.5° F within the three months. A steady, though gradual, increase in the maximum and minimum temperature occurs as the dry season begins to merge into the wet season. In the shaded portions of the breeding area it often becomes cool enough at night to demand a blanket for cover, i. e., should one care to sleep there. During the daytime this same locality has a very moist heat, hard to endure.

The great regulator of temperature is the aqueous vapor, (Abbot 1899) "as it is less permeable than dry air to the waves of energy from the sun and still less so to those that radiate from the earth. Its influence in this direction is very important on the Isthmus of Panama because there is only a narrow strip of land between two great oceans, and consequently the relative humidity is always very high. By combining high temperatures with this high humidity there results an excessive absolute amount of moisture in the atmosphere."

C. Rainfall. In humid sections of the torrid zone rainfall is such an important factor of the environment, that a table showing the annual rainfall for three years for the various stations of the Canal Zone is inserted. The average annual rainfall for Gatun for the past nine years has been 129.30 inches, and since the rainfall for 1913 was but 112.81 inches, and that of the two years previous still less, it follows that there were years when the rainfall exceeded 130 inches. In 1913 there were 248 rainy days at Gatun, leaving but 117 days with no rain, i. e., less than four months of clear weather.

Rainfall of such character exerts great influence upon the environment and its biota. With heat and wind it regulates the environment.

E. Annual Rainfall for Three Years.

Station	1911	1912	1913	Station Average	Years of Record	Rainy Days '13
Ancon	64.14	71.78	65.98	70.90	16	180
Balboa	63.73	71.89	59.54	69.86	15	169
Mira Flores	61.97	88.49	70.12	87.33	5	183
Pedro Miguel	64.12	75.71	69.65	82.32	6	180
Rio Grande	82.11	75.14	64.51	86.13	9	199
Culebra	74.84	78.99	69.09	88.78	23	195
Comacho	84.72	77.98	73.79	91.46	7	190
Empire	66.70	74.56	74.78	80.43	9	196
Gamboa	70.67	89.07	86.28	92.65	31	207
Juan Mina	82.46	88.24	77.13	87.04	3	190
Alhajuela	90.05	83.73	77.41	102.43	14	196
El Vigia	82.64	94.65	77.13	105.22	5	197
Frijolles	.....	104.66	109.34	107.01	2	243
Trinidad	91.53	103.04	97.27	117.03	6	258
Monte Lirio	113.27	100.74	107.58	129.75	6	177
Gatun	99.28	111.83	112.81	129.30	9	248
Brazos Brook	116.08	124.66	138.89	138.64	7	260
Colon	112.75	117.59	131.22	129.38	43	246
Porto Bello	148.94	147.61	171.19	169.15	6	272

The daily rainfall at Gatun from September, 1912 to September, 1913 is given in table "F". The dry season months of January, February and March show but 38 rainy days out of the 90 of this period. But the rainfall has been less than the average for the station. Only on three days had there been rainfall in excess of one inch. The rains nearly always fall during the afternoon, but it is not unusual to have a mean, drizzling rain all day long, augmented by a changing wind. Thunder storms or violent rains are rare.

F. Daily Rainfall, Gatun, 1912–1913.

Automatic register; in inches. Values midnight to midnight.

Date	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
1	.02	.07	.02	.01	.20	.06	.02	.03	.01	1.70	3.04	0
2	2.45	.38	1.58	.01	.15	.04	.09	.01	1.51	.38	.07	0
3	.96	.28	.08	.42	.22	.02	0	.01	.10	.74	0	.06
4	.18	.12	1.56	.01	.04	0	0	0	0	.62	.46	0
5	.43	.11	.99	0	.44	0	0	.06	0	1.29	.63	.17
6	0	1.02	1.18	0	0	0	T	0	0	.40	.36	1.62
7	.11	2.04	.35	0	0	0	0	0	.13	0	.06	0
8	.25	.26	.31	0	.12	0	.09	0	.14	0	.18	.08
9	0	.02	.44	.30	0	0	.15	0	.22	0	.43	.14
10	.03	.06	.96	.26	0	0	0	0	.06	.06	.02	3.04
11	.08	.14	1.32	.08	0	.01	0	0	.18	1.55	0	.07
12	0	0	.01	.51	0	0	T	0	1.43	1.47	0	.44
13	.01	0	0	.01	0	0	0	0	1.30	.76	0	0
14	1.14	.23	.07	.13	.02	.13	0	0	3.19	.47	.11	0
15	.01	.07	.04	1.79	0	.02	0	2.06	.94	0	.69	.45
16	.01	.56	.08	2.52	.21	.09	0	.20	.16	0	.08	.18
17	0	0	.14	0	.42	1.42	.03	0	.25	.36	.20	.35
18	0	1.06	.95	.03	0	1.07	0	.07	.06	.01	.19	0
19	0	1.31	.35	1.34	.04	.01	.01	0	.27	.14	.02	0
20	.07	1.60	.58	0	.01	0	0	0	.96	.02	0	.18
21	1.49	.43	.53	.06	0	0	T	0	.41	.02	0	.55
22	0	.02	.06	.10	0	0	.18	.05	.71	0	.04	2.30
23	.07	.37	.17	.06	.09	0	.03	0	1.39	.09	1.42	.27
24	.04	0	.67	.45	0	0	0	.86	1.77	0	.03	.05
25	.08	0	.15	.16	0	T	.17	.64	.14	0	.04	.10
26	.01	1.73	.24	0	0	0	0	.58	.51	.13	.02	.21
27	.39	1.05	1.63	.03	.23	.05	.18	.08	.51	.42	.85	.09
28	0	0	1.54	.26	1.71	T	.01	.06	.02	0	.42	1.78
29	0	.32	1.53	.02	0	.....	.05	.09	.39	.05	.27	.15
30	.01	.72	1.65	.16	0	.....	0	.58	.29	.02	.10	.01
31	.....	.55	.....	1.10	.73	.....	0	.....	.01	.....	0	.03
Total 1912	7.84	14.52	19.18	9.82	4.63	2.92	1.01	5.38	17.06	10.70	9.73	12.32
Total 1913	4.33	16.92	15.78	2.25	.91	2.38	.55	4.18	13.83	14.80	11.84	11.98
Stat'n Avr.	9.70	16.62	22.40	13.38	3.92	2.46	2.70	4.20	15.82	13.39	12.08	14.09
Excess ordefi.	—1.86	—2.10	—3.22	—3.56	+.71	+.46	—1.69	+1.18	+1.24	—2.69	—2.35	—1.77
Rainy days	21	25	29	24	15	11	12	15	28	21	24	23

D. Humidity. This is the product of heat, wind and water. Humidity is always high on the Zone, though lowest during the dry season. The following table gives the Mean Relative Humidity for the three sections of the Zone, for a period of one year. In the region of Gatun the lowest point reached was 78%, from February to May, and the highest was 89%. Humidity is a powerful agent in the regulation of breeding periods, life duration, time of activity, etc.

G. Mean Relative Humidity (%) 1912-1913.

	Sep.	Oct.	Nov	Dec.	Jan.	Feb.	Mar	Apr.	May	June	July	Aug.
Ancon (Pacific section).....	91	93	92	89	87	83	78	76	88	89	90	91
Culebra (Central section).....	93	93	93	91	89	86	82	80	91	91	91	94
Colon (Atlantic section).....	87	88	89	84	82	78	78	78	87	88	87	89

E. Fogs and Cloudiness. Fogs at Gatun are not numerous and such as do occur are nearly all dissipated by 6:30 a. m., and all by 8:30 a. m. As the dry season advances, night fogs are fewer in number. Observations at Culebra, Canal Zone, where fogs are of almost daily occurrence, tend to show that they impede the flight of mosquitos. But at Gatun the fogs are all light, not common, and in no way seemed to interfere with the flight of mosquitos.

The dry season is marked by a general absence of clouds, and such as do exist, appear usually in the afternoon. Data is lacking as to what bearing clouds may have upon the environment or organism, but it appears they cannot exert a big influence or else it would have been noted.

F. Seasonal Changes. The general weather conditions for 1913 are expressed in the following chart:

H. Weather Conditions, Canal Zone, 1913.

Rainfall deficient (except Brazos Brook, Colon and Porto Bello).
Dry season rainfall:
Pacific section=4% total.
Central section=6% total.
Atlantic section=10% total.
March least rain. May most rain.
Air temperature and wind movement slightly above normal.
Atmospheric pressure and cloudiness generally deficient.

	Pressure (reduced to mean of 24 hrs.)	Temperature					Mean realtive humidity	Precipitation			Wind Movement				
		Mean	Max.	Date	Min.	Date		Total In.	Station Average	No. of Rainy days	Avr. hourly Movement (Miles)	Prevailing Direction	Max. Vel. (Mi.)	Direction	Date
Colon...	29.866	80.1	91	Jun. 22	71	Feb. 4	85	131.22	129.38	246	10.7	N	36	N	Nov.14
Culebra	29.846	79.2	95	Apr. 14	64	Jan. 4	90	69.09	88.78	195	7.3	NW	40	N E	Nov.19
Ancon..	29.834	80.3	96	Apr. 27	66	Feb.22	87	65.98	70.90	180	7.2	NW	32	S	Jun. 11

At a given station, seasonal variation is not marked. But it is evident that at Ancon, where rainy days are one third less than at Colon, and rainfall less than one-half, that a different set of conditions are present there than at Colon.

It is interesting to note that the rainfall for December '12, was generally deficient throughout the Zone, but that at the Colon-Gatun section it was much heavier. Thus the swamp near Gatun which caused so much *Anopheles* breeding in the early part of 1913, was prepared for such wide-spread breeding by this increased rain. The influx of pure water meant that the salts in the swamp, which rapid evaporation was about to make stronger, were kept diluted and never became so strong as to inhibit mosquito breeding. It meant also that the density of the water was lowered.

In January, 1913, temperature and relative humidity were above the average. February continued with deficient wind movement. March and April showed also a deficiency in relative humidity and atmospheric pressure. In May these conditions reached their normal. Cloudiness had been deficient throughout the dry season.

G. Composition of Water. By order of the Chief Sanitary Inspector, daily samples of water from the salt-marsh were sent to the Ancon Hospital laboratory for determination of salt content. After eliminating such samples as appeared untrustworthy, the following table was prepared. All samples were from water where larvæ were numerous.

J. Chlorine Content of Salt-marsh Water.

Sea-water = 22,000 parts of Cl per million 0.022%
Port Limon water = 19,000 parts per million 0.019%

MAXIMUM.

	Parts of Cl per Million	= % of Sea water	= % of Port water
April 8	23,500	107.0	123.7
April 2	21,000	95.0	110.0
May 7	19,083	86.0	100.0
May 8	18,833	85.0	99.0
Feb. 26	18,500	84.9	97.4
Avr.	20,183	90.0	105.0

MINIMUM.

Feb. 11	11,250	51.1	60.0
Feb. 12	12,150	60.0	64.0
Feb. 3	12,500	60.0	66.0
March 8	13,500	61.0	71.0
Feb. 6	14,000	63.6	73.0
Avr.	12,680	57.6	65.0

General Average:

Samples	Days	Total Cl	Avr. per Million	% sea-water	% Limon water
38	27	617,480	15,817	72.0	83.0

It is seen that larvæ were breeding in water containing from 55% to 107% of ocean salinity, i. e., even in water more saline than the ocean. The density of sea-water is about 2.5% greater than that of fresh water, and this added buoyancy probably is to the advantage of such larvæ as can thrive in salt waters. Had the salt content been greater, the buoyancy would be too great for the larvæ, besides, the fact that the irritation due to the chemical content would inhibit any extensive breeding.

Due to the absence of hills, rains could wash no silt into the breeding area, and what was present, settled to the bottom. Imperfect drainage prevented currents, though in time stagnation would follow and make unfit the environment. The latter part of January, 1913, a ditch was cut through the swamp, and shortly after a pipe-line dredge began to pump silt into the swamp. This violent disorder in the environment—current, silt and drainage—soon began to tell upon the numbers of mosquitos breeding in the swamp.

II. Biotic Factors.

The microbiology of the waters of the marsh was not studied. *Spirogyra* and *Oscillaria* were found within the digestive tract of larvæ of *A. tarsimaculata* and *Aedes taeniorhynchus*, but these green algæ can hardly be the only source of food since larvæ of both species have been found in situations devoid entirely of algal growth. The decaying leaves and fallen twigs, excreta of animals and decaying animal matter favor the growth of micro-organisms, and these probably are eaten by the mosquito larvæ.

Trees and shrubbery are important factors because they furnish the needed protection from intense light and heat, and lower the rate of evaporation. So important is this protection to the adult that one never sees these small flies active in the open during the daytime. However, hunger often changes existant physiological states, and so the presence of a man in an exposed place near which mosquitos are hidden, may often bring these toward him in great number; and the first act to be noted is an attempt to sink their proboscis into flesh. But if no human being is present, the mosquitos remain in seclusion. When so venturing forth to secure a blood meal, they show no negative reaction toward heat or light, and may even suffer body mutilations without evident consciousness of pain.

No effort was made to survey the marsh for the animal life it contained, but birds were noted to be the most numerous next to insects, followed by snakes, lizards, iguanas, monkeys and armadillos, the last two rather uncommon. Cows occasionally strayed into the area and when examined on one occasion, were found to have their ears well lined with busy *Anopheles* and *Aedes*.

A species of night-jars (Fam. Caprimulgidæ) has been noted repeatedly at dusk, flying low over the old French Canal, and their actions were those of feeding. This lasted for about an hour, and coincided with the period of mosquito flight. These activities were also noted during dawn, again when mosquito flight was in progress. These night-jars often shifted their position along the Canal, and by row-boat observations it was found that they were where mosquitos were thickest. A shot gun brought down three of these birds and their gullets contained adults of Anophelenes. The stomachs contained in addition ants, hemiptera and a few coleoptera. That these birds were feeding upon the mosquitos is undoubted, but the effect of their ravages was not significant for there seemed to be an infinite source of supply for these mosquitos. Jennings (1908) records a similar case in the Bahamas, the species involved being *Chordeiles virginianus minor*.

The blood available to mosquitos at the marsh is only such as they can get out of cows, monkeys, birds, lizards, etc., and this is not enough. The fact that these mosquitos flew a mile or more each night to secure rich, human blood, places man among the most important of the biotic factors entering into the mosquito environment. Since the winds from the marsh have been almost wholly from the north or northwest, and not one hour from the southeast, it cannot be argued that the scent of man was born to these mosquitos by the wind and all they had to do was to follow the trail. These mosquitos flew as an air-man does, at a quarter to the wind, and they flew till they found food; it is not at all improbable that they would have flown five miles if Gatun were so distant. Their flight was in quest of food. Here is an example of an animal whose environment is quite scattered.

The question of whether a blood meal is required, or if the mosquitos can live on the juices of fruits, must be answered separately for each species. Of the species treated in this paper, there is no doubt but that they prefer human blood if that is available, and will struggle against many odds in order to get it. Darling (1912) found he could keep adults of *Aedes calopus* Meigen alive 110 days in captivity by means of raisins and ripe bananas, and *Anopheles albimanus* alive 12.5 days. The author has noted on several occasions *Aedes taeniorhynchus* Wiede. feeding on ripe bananas, and once, while searching

for Thysanoptera, found a male of this species inside of a flower. But if the female mosquitos of these species do eat fruit juices under natural conditions, it appears only fair to believe some one of the able sanitary corp of the Isthmus would have made a few observations of the fact. The infrequency of such observations is explainable on the presumption that human blood is preferred and so soon as a human being is anywhere near, his presence is quickly detected and sought long before he could have found out the whereabouts and doings of these pests. This at least is true of the half dozen common species on the Zone.

Jennings (1912) in his survey of the upper Chagres River valley, did not encounter adults of *A. albimanus* Wiede. nor larvæ, though habitats were seen which if present on the Zone would favor *Anopheles* breeding. He attributes this absence to the absence of habitations, a presumption fairly accurate. Busck and Orenstein made a trip to the Upper Trinidad valley near Gatun, and but one *albimanus* was collected by them, though the sylvan *Anopheles* were abundant. (There is some doubt as to the authenticity of this single *albimanus* as it may be an accidental mix-up with mosquitos from the Zone.) The writer in his inspections of the Canal Zone, found *albimanus* to breed only near settlements. It therefore seems quite plausible to believe that the pathogenic species of *Anopheles* become more and more restricted to human settlements, an adaptation which no doubt will hold for all animals which play a role similar to that of *albimanus* in the transmission of disease. This trend is probably due to repeated feedings upon human blood, and it may be that the development and establishment of the malarial parasite within the mosquito may have had a tendency toward such isolation. The restricted distribution of *Aedes calopus* tends to strengthen the idea that pathogenic species cling to inhabited regions.

It also appears that a meal is necessary prior to oviposition. The studies of Darling (1912) indicate such to be the case. The author (1913-c) recorded a case of oviposition in *Aedes calopus* where prior meal was absent. The fact that the mosquitos concerned in the flight to Gatun returned daily to the marsh, would indicate that food and oviposition were closely linked together. It appears only natural that a mosquito upon emergence from its pupal prison, should seek, first

of all, food. While it is true it does not increase in size after emergence excepting as food or eggs swell the abdomen, it does seem that the reproductive organs need further growth, and for this food must be taken. But since the preservation of the species becomes a powerful factor at work within the animal, so starvation and captivity may cause a hurried development of ova. In his dissection of gravid typhoid flies, the writer found more ova in flies which had been fed after emergence from the puparium than in flies totally deprived of food.

The drastic anti-malarial measures of man place him a powerful agent of destruction at work in the environment. Man as an agent hastens or retards natural processes. Thus by means of a ditch and a pipe-line dredge, he has driven the mosquito from its paradise and made its return thereto impossible.

III. Historic Factors.

The creation of the salt-marsh habitat can be traced to the work of the old French Canal Company, as already described. When Americans built the big Gatun dam and made the Spillway, they did more than impound the waters of the Chagres. They changed the drainage, tamed the river which often came down in flooding proportions, and by allowing it to peacefully flow past the new Spillway, did away with the annual floods. The floods eliminated, and the increased rainfall in December, 1912, were the prime factors which prepared the marsh for extensive breeding.

B. DYNAMICS OF THE ENVIRONMENT.

I. The Habitat of the Immature Stages.

Larvæ and pupæ were found most frequently associated with green algæ, which plants afford them ample shelter, support and fair protection from fish and larvæ of carnivorous insects. The respiratory tubes of the larvæ were often noted in close proximity to the bubbles of oxygen given off by the algæ. Experimentally, young larvæ (2d moult) were sealed hermetically in a glass jar containing filtered water from the marsh and a small quantity of living algæ; the larvæ developed into adults. Out of the ten larvæ originally placed in the jar, three adults ensued. The time duration was almost twice that under

normal conditions, but it is clear that growth was maintained because of the exchange of the voided products of respiration by both plant and animal. The balance was easily destroyed by having too many larvæ or too much algæ. This sort of interrelation between organisms is very close in the forms studied, and therein are found the greatest number of points of contact with the environment than elsewhere. Any new factor, or one which is present but is exerting undue activity, stretches the relative balance which existed in the association, and the response caused thereby on the part of the members of the association will be in the direction of the establishment again of a new relative balance. It was quite evident from many observations made that in many ways the larval association was susceptible to quick destruction from such external causes as the presence of silt, wave action, etc.

It was stated that as the dry season approached, the winds increased in velocity, the heat became more intense, and rainfall decreased. The resulting rapid evaporation concentrates the salts in the water of the marsh, changes thereby the density of the water, followed by a change in the microbiology of such a habitat. Such changes usually bring about less mosquito breeding, and on the Canal Zone for many years it has been observed that during the dry season *Anopheles pseudopunctipennis* Theob. is the dominant Anophelene—a non-transmitter of malaria. The dominant rainy season Anophelene is *albimanus*. This change in the mosquito fauna is due to habitat changes brought about by the change in the climatic factors. During November and December, 1912, and January, 1913, there was an increase of 14.7 inches of rainfall over the same period the year previous. This increase of pure water at the swamp was sufficient to dilute the salty water to such a degree that subsequent evaporation did not increase the salt-content above a density inhibiting mosquito-breeding. This supposition is strengthened by the fact that in previous years, conditions exactly alike excepting for this increased rainfall during the indicated months, this area caused no influx of mosquitos into Gatun.

Reference was made to the fact that the larvæ from this swamp developed in water which equaled sea-water in chlorine content. The density of such water is 2.5 greater than that of fresh water—a difference sufficient to float chewing gum. Such

added buoyancy reduces the muscular effort needed to reach the surface, and probably reduces the mortality due to fatigue among the larvæ.

At the laboratory, larvæ of *A. tarsimaculata* taken from fresh water were transferred into a pan containing saline water from the marsh. This produced intense stimulation and a large mortality resulted. Pupation was accelerated among mature larvæ. The adults that emerged were placed into a large cage containing a plate of fresh water and a plate of salt water, both from actual habitats. A liberal blood meal was given. Eggs were found only in the plate with fresh water. Continuing the experiment, but using pupæ collected at the salt marsh habitat, eggs were deposited in both salt and fresh water. There appears to be a natural selection as to water suitable for oviposition, and in nature such must be the case, for many bodies of water are encountered which appear excellent breeding areas, yet are found wholly devoid of larvæ. At the same time the entire surrounding territory may be literally alive with Anophelenes. When very young larvæ are thrust into saline water, attunement to the rapid change is quicker than when older larvæ are so treated.

The general belief is that *Anopheles* will not breed in salt water. This is because the average observer fails to learn that the genus *Anopheles* contains a large number of species which can be grouped into several very distinct sections. The chances are if all Anophelenes bred in the same kind of waters, we would have a far fewer number of species.

DeVogel (1907) found a species of *Anopheles* breeding in a pool containing 2.8% of NaCl; and after considerable study, he arrived at the conclusion that the eggs of species which can live very well in sea-water, develop in such water even when evaporated to one-half its original bulk, but that the larvæ do not appear to transform to adults if the concentration exceeded 33% of the original quantity. This in the main part is also true of *A. tarsimaculata* on the Canal Zone. Banks (1908) found *Anopheles ludlowii* Theob. breeding in both salt and fresh water, quite like *tarsimaculata*. Howard, Dyar and Knab (1913) criticize Banks' work as inconclusive and believe his fresh-water *ludlowii* was none other than *A. rosii*, and there is ample room for such doubt since determinations were made only from larvæ—a difficult task.

The excrementa of animals living in the swamp, the decay of leaves, branches, carcasses, etc., produce local changes which may prove destructive to larvæ. These larvæ nearly always manage to wriggle away from the influence of such pollution and these chemical changes hardly ever become general enough during the cycle period of the mosquito so as to exterminate all breeding. Even the admixtures of oils and larvacides are found to be difficult, for it is practically impossible to give thorough treatment in all places. The total pollution for the entire season can hardly make the environment unfit for mosquito larvæ. The heavy rains of the next rainy season will again readjust the environment; therefore such pollution can hardly become accumulative.

II. The Habitat of the Adult.

The hottest and windiest part of the day is about at noontime, and is the period of least activity in the animal world. It is during dawn and dusk that mosquitos sally forth and show pronounced activity. During the daytime they are hidden in the bush, under buildings, etc. The factors that regulate this time-adaptation are light, heat and wind. Among the factors which disturb the tranquility of the mosquito world, man and his radical measures assume greatest importance. Ditches, oil, hydraulic fills and plenty of patience are the tools that slowly but surely get at the root of such serious breeding and convert it to unfitness for such purpose.

DYNAMIC RELATIONS OF THE MOSQUITOS.

A. REVIEW OF THE SALT-MARSH BREEDING AREA.

The early part of January, 1913, the writer was sent to Gatun. The conditions found were really alarming, inasmuch as the species involved was a malaria-transmitter, and the unscreened, congested town of New Gatun invited a spread of malaria. This influx of mosquitos was unexpected and hence unprovided for with funds, but the energetic work of LePrince and Corrigan, aided by several sanitary inspectors, soon brought this danger within control. The following table is introduced to show the number of mosquitos actually caught during the three weekly periods ending February 15, 1913, and the number of reported cases of malaria for the same period.

K. Anopheles and Malaria, Gatun District.

	Feb. 1	Feb. 8	Feb. 15
Trap catch, Gatun	1,039	604	387
Trap and hand catch, both Gatun and New Gatun	12,067	11,897	12,838
Total	13,106	12,501	13,225
Malaria Cases, American Whites	5	4	8
Malaria Cases, Gatun, all others	16	20	19
Malaria Cases, New Gatun	31	33	39

By trap catch is meant those mosquitos which were caught in the "C. H. Bath" mosquito traps attached to barracks, and hand catch refers to mosquitos caught by expert negroes within buildings, by means of a small killing vial. All counts were made daily.

Though the white American is presumed to be more susceptible to infection, his well-screened home, his rather regular habits, and the free use he makes of the dispensaries, protect him amply against ready infection. The cases reported are mostly of men working on night shifts. Negroes and Spaniards show more malaria, due largely to habitual loungings outdoors after dusk, thus exposing themselves to *Anopheles* bites. The New Gatun high percentage is due to lack of screening (Orenstein, 1912-b). The New Gatun cases, as well as those of negroes in Gatun proper, are only such as presented themselves at the dispensary, and often malaria was a secondary diagnosis, for the patient complained of totally different symptoms. It is the general rule that the first attack of malaria gives the negro considerable fever, whereas subsequent attacks are much milder. Very often a negro may have a good quantity of parasites roaming about in his blood, yet be unconscious of the fact. Such cases do not find their way into the dispensaries, and of course are among our worst enemies, for they allow the spread of the fever. Therefore it may be reasonably supposed that the malarial rate in New Gatun was at least fifty per cent. higher than reported.

The narrowness of the strip of the Canal Zone, its congested labor centers, frequent trains and heavy traffic, added a big factor, that of the spreading of *Anopheles* from this place to

other stations. Adults of *tarsimaculata* have been picked up in passenger and freight cars forty miles from Gatun, and later in the season larvae were found at Balboa and Ancon which upon maturity yielded *tarsimaculata*. There is no doubt whatever in the writer's mind as to the origin of this new breeding area; the invaders in these swamps of Ancon and Balboa came from the Gatun influx. The writer examined at regular intervals the mosquito catch from Monte Lirio and Frijolles, seven and thirteen miles distant from Gatun, and at first the *Anopheles* were wholly *albimanus* and *malefactor*, but toward the end of February, *tarsimaculata* was not at all uncommon.

To outline briefly the history of the breeding at Gatun, it was generally supposed by several inspectors that the trouble came from the floating islands and vegetation in Gatun lake. An inspection from a launch revealed nothing. Mr. J. A. Corrigan, the inspector in charge had a totally different idea, and after the others returned without results, he invited them to the actual source of trouble, the salt-marsh north-west of Gatun. How he came to that conclusion is probably explained on the basis that he knew not merely his district, but also the territory just outside of it. At any rate, once well within the thickets in this marsh, all agreed it was as near true to the most authentic descriptions of Hades as could be had.

Nor was this tormenting scourge confined to the marsh. The early part of January, the clerks in the Administration building at Gatun began to place blotters on the seats of their wicker chairs; some claimed this measure only alleviated the discomforts. Colonel Phillips came to investigate, and left the place with an *Anopheles* clinging to his coat, and this mosquito held on notwithstanding that a ten-mile wind flapped violently the coat. The writer noted on March 20th, a. m., a negro with a female *tarsimaculata* resting on his coat, and traced it over two hundred yards. In a previous paper (1913) the writer reported having carried *A. albimanus* into Corozal which had been clinging to his clothes for quite a distance. *Anopheles* cling more tenaciously to clothing than do *Culex*.

At the hotel, particularly at night, mosquitos insisted in painfully assisting the men trying to eat. The location of the door, as well as the fact that it was practically continuously open during the hours when mosquito activity commenced,

was the real cause of so many of these pests being within. Once in, and finding the electric lights too intense, they remained under the tables. The Y. M. C. A., close to the hotel was relatively free from mosquitos, due to the fact that the door was not situated the same as the hotel door.

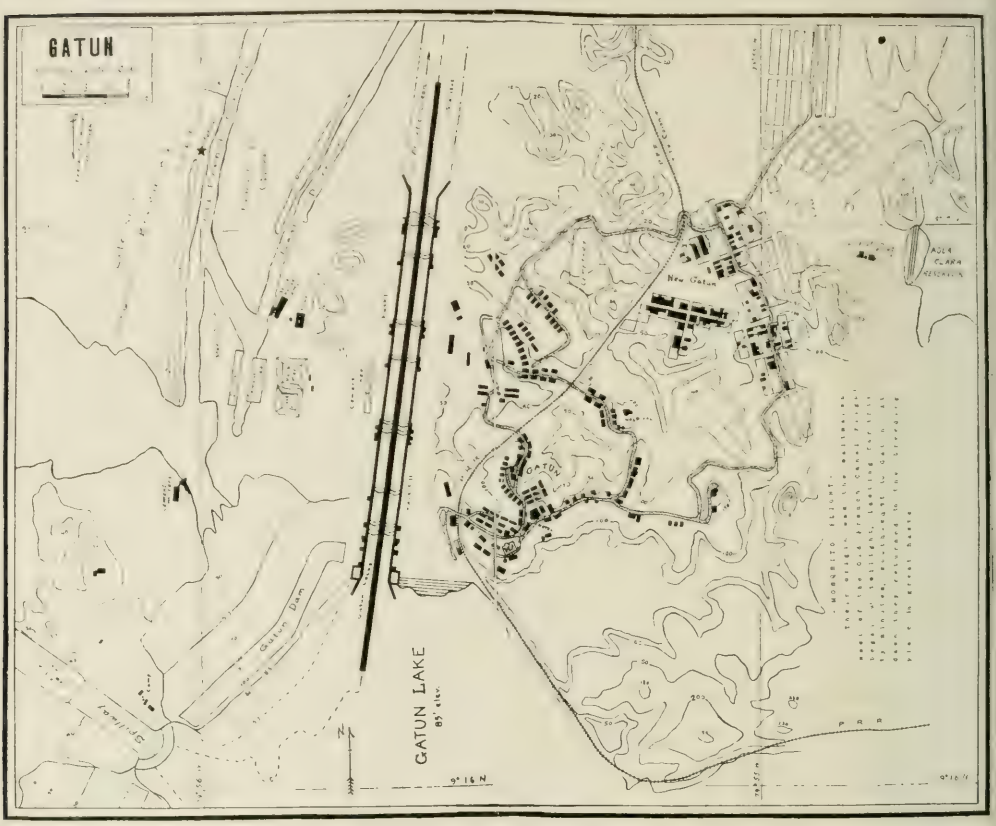
It is impossible to estimate the number of mosquitos which paid daily visits to Gatun, and were any true statement made, it would appear fictitious. At the cement shed, close to the breeding place, cob-webs were so heavily loaded with mosquitos that they sagged and were torn in places, (Zetek 1913-b). The presence of so many mosquitos in these cob-webs is explainable by the fact that mosquitos inside of the building always aim to get out at dawn, at least so has been found to be the case over and again, and there being many cob-webs about the windows, large numbers of mosquitos were caught in them. The spiders do not seem to care for mosquitos. To be stranded at the breeding place was a most painful trial, though unusually fruitful in results.

Sweep-net catches showed mosquitos harboring in the low grass whenever the day was foggy or cloudy, but in clear, warm days, but few were found in such grass. Cracks in the soil were found to harbor *Anopheles* (also black flies). The shaking of bushes near the breeding place frightened away veritable clouds of *Anopheles* and *Aedes*. By placing a negro within a mosquito bar net having one side slightly raised and exposing same during dusk (at the breeding place), there were attracted into that net a trifle over three thousand *Anopheles*, actual count, which represents about a hundred mosquitos entering the net each minute.

Ocular inspection at the right time of the day—at dusk and during dawn—sufficed to detect a noticeable flight of the mosquitos to Gatun from the salt-marsh. Marking the adults with anilin dyes was done only to clinch the facts, to prove beyond all doubt that the mosquitos seen in flight really were bound for Gatun.

The remedial measures pursued to stop this immense breeding were the digging of two drainage ditches and the reclamation of the marsh by hydraulic fill. The trees and jungle at the peninsula fronting the breeding area were cut and burned over by February 4, 1913 and the smoke and the destruction of a large area of shelter were effective in reducing for the

time being the numbers of mosquitos flying over this section. However, observations made tend to indicate that the path of flight was changed since the area was burned over, as if to avoid the barren waste. Mosquitos harboring in the brush were killed when this vegetation was burned, but these mosquitos were only a very small fraction of the entire horde and



hence their destruction amounted to very little. Ditch "A" (see map) was finished toward the end of January, and the swift current of water through it made an ideal habitat for the sand flies which during February became very numerous. Ditch "B," south-west of "A," was completed early in February. On the 18th of February, a dredge began to pour silt into the marsh, at a point about 4,000 feet to the north of ditch "A." On February 27th, the outlet of this discharge

pipe was moved in the direction of ditch "B." In places the pupae were so thick as to give the appearance of heavy moss. At regular periods large areas of very young larvae were encountered, indicating that breeding was far from stopped. On March 13th, about four gallons of top minnows were taken from the old French Canal and transferred to the marsh, but these fish left no record of having in any way checked the numbers of larvæ.

The chlorine content of the water exceeded at times that of sea-water. When the fine silt began to mix with the water, the beginning of the end was plainly in view. Ditch "B" was deepened, while "A" was closed. This allowed the fill to proceed toward "B." By the end of June the paucity of larvæ was very noticeable and during July and the following months no breeding was encountered.

B. LIFE HISTORY.

1. *The Immature Stages:* The flight experiments and the daily examination of thousands of mosquitos, allowed but little time for a study of larvæ and pupæ. The duration of the pre-adult stages of *A. tarsimaculata* were found to be as follows: egg-instar fifteen to twenty-four hours; larval stage from four to five days; pupal stage from two to three days; or, for the entire period, seven to nine days. *Aedes taeniorhynchus* from the same locality matured about a day sooner. No distinguishing markings could be made out in the field with a 10x lens which separate true *albimanus* from *tarsimaculata*. The pupæ of the latter appeared to be darker in color, but from a series of pupæ from other stations, this color difference proved to be valueless. To the sanitary inspector it is enough to group the species into several easily recognized divisions. Thus on the Canal Zone, the Anophelenes may be divided into (1) the *albimanus* group; (2) the *malefactor* group; and (3) the *pseudopunctipennis* group.

Larvæ orient themselves readily to regions of shade. Sunlight, or any highly intense light, which may shine directly upon a mass of larvæ, causes great commotion among them, which excitement finally ends in shaded portions of the pool. A similar reaction ensues when a film of vegetable oil reaches larvæ. There is also a decided reaction to shadows that pass

over a body of larvæ, in this case the movement is downward and not transverse. They remain quietly on the bottom for several minutes. Pupæ are the more restless, and cannot remain below the surface as long as do the larvæ. When the pipe line dredge poured silt into the marsh, larvæ were not found in the silt-laden waters, but always just in front of it. When silt became generally distributed, the reduction of larvæ was very pronounced.

At the laboratory several tests were made to learn the reactions to such stimuli as gases passed over the surface of water containing larvæ. A large basin was used; it contained pupæ and larvæ and water from the breeding place. Three glass jets of about 1 mm. diameter and 2 cm. apart, were placed 2.5 cm. above the surface of the water, at the middle of the basin, and in such a way that the streams of gases passing through these jets shall pass parallel to the surface of the water. The gases used were oxygen, carbon-dioxide, hydrogen sulphide and chlorine. Each test consisted of five minutes of constant stream of gas. Two hours elapsed between each test. There was no marked reaction toward oxygen. When CO_2 was used, no reaction was noted during the first two minutes, but after that all larvæ slowly separated toward the two ends. This test was repeated several times, varying each time the light at both ends, but the reactions were alike. Similar separation occurred with H_2S and Cl, being most marked and almost instantaneous with the latter.

Similar experiments were made, excepting that the gases were allowed to diffuse in the water. The modifications in apparatus consisted in the addition of a small fan-shaped jet near each end through which fresh water flowed into the basin; opposite these two jets were small outlets for overflows. At the middle was a small fan-shaped jet through which the gasses passed. All gases were introduced for periods of five minutes, and a new lot of larvæ and water were used for each test. The flow of gas was regulated so as not to agitate the water. The number of larvæ were never less than a hundred.

When oxygen was introduced, the larvæ nearest the stream of gas became more active, but except for this, no other reaction was observed. With carbon-dioxide the larvæ separated for both ends, and at the end of 4.5 minutes they wriggled to the

bottom where they remained almost motionless. None came to the surface until thirty minutes after the stream was cut off. Chlorine and bromine gases had very decided effects; immediately the gas was introduced the larvæ began to wriggle violently, and after the first minute slowly lost all motion. Some remained at the surface, others at the bottom. Five minutes of either gas was sufficient to kill all larvæ at the end of one hour and ten minutes exposure in the water.

Successful introduction of a gas into water breeding mosquitos would kill all larvæ in short time and would prove a valuable agent in the reduction of breeding. But from the fact that the average native is not over-careful as to the kind of water he drinks, extreme caution must be used in the gas used. Under certain conditions it would be quite effective to introduce suitable electrodes into saline waters breeding mosquitos and destroy these by the liberation of chlorine gas through electrolysis.

2. *The Adult Stage:* Elsewhere it had been stated that the food of the female *Anopheles* transmitting malaria is probably restricted to human blood and that this exclusive diet was reached by a gradual adaptation and restriction to the human race, covering probably centuries of time, and probably at some period thereof has been accelerated by the introduction and establishment of the malarial parasite. That such mosquitos are restricting themselves to inhabited regions is clearly in evidence on the Canal Zone. Regions away from habitations have habitats which are identical in every respect to those on the Zone which breed *albimanus*, and yet they are sterile as to such species. Nor are there adults of such species in the bush nearby. In the malarial mosquitos this restricted adaptation is not as complete as that of the yellow-fever mosquito, *Aedes calopus* Meigen, but an unmistakable trend in the direction of greater restriction is plainly in evidence. Females predominate by great percentage in all traps attached to buildings and in mosquitos caught inside of buildings. The proportion is as high as 250 : 1. Smears of the stomach contents of over a hundred males caught in Gatun barracks showed no case of a human blood meal. It is also quite probable that if males did suck human blood, some member of the sanitary corps would have noticed instances of it. It seems that the male *Anopheles*

does not suck human blood. Occasionally females are found with the antennæ quite plumose, so much so as to resemble males at first sight. Therefore all supposed males seen sucking blood should be caught and examined carefully as to sex.

Observations at Gatun suggest that a blood meal is necessary before normal oviposition can take place. Darling (1910 p.27), records a case of a virgin *Aedes calopus* with developed ova after it had but one meal. In another place he states that the eggs from virgins are sterile. In the laboratory, freshly emerged *tarsimaculata* did not copulate during the first two days when deprived of all food, but a few did so on the third day. When a meal was given the first day, copulation was noted early the next day. Dissection of females three days old showed the ovaries most developed in those individuals which had had food. The daily flights to Gatun were in quest of food, and the morning return flight was most probably for the purpose of oviposition. That it was not for shelter is evident since abundant shelter is found under buildings in Gatun, and this shelter was made use of by large numbers of mosquitos. It appears from the few observations made that copulation takes place in the air, during the morning return flight. Occasional males were picked up in the flight to Gatun, but always near the breeding place and during the morning return flight. More males were noted at the return flight than in the evening one.

Laboratory experiments indicate that after a good blood meal an *Anopheles* does not bite again until after the pellet of waste is evacuated. When no blood meal was available, *Anopheles* were seen to suck water. Usually after a meal on ripe banana, a drink of water was taken.

Kept in a screen cage of about two cubic feet, and controlling light, heat and moisture as much as possible, with a few crushed raisins as food, adult *tarsimaculata* were kept alive twenty-six lays. How long they may live outdoors cannot be said, however, it appears certain that they cannot be as long-lived as are some of the mosquitos of colder climate. The severity of heat and moisture lower the life duration period, and the depreciation brought about by this check is counterbalanced by increased reproduction and more rapid development of the pre-adult stages.

Reference was made to the habits of the night-jars*, which appeared when mosquitos were in flight and fed on them. The numbers thus eaten are not large. The main food of these birds consists of hemiptera and ants. Bats were noted only sparsely, while tree frogs, spiders and lizards were seen frequently catching Anopheles. By far the most destructive agent in the mosquito's environment is man and his various measures.

The effect of oils rubbed on the skin, as a repellent to *Anopheles*, was tried out at the marsh, but the negroes who smeared themselves concluded the oils were more bothersome than the mosquitos. Pyrethrum was also burned. The method used was to place a negro into each of the four mosquito-bar nets and after about two hours of exposure, the nets were tied at the bottoms, placed into a closed room and the mosquitos killed with sulphur dioxide. The tent with burning pyrethrum was the furthest away so that the wind would not carry the fumes to the other tents.

	Oil of Sassafras	Oil of Creosote	Pyrethrum	Check
Albimanus	6	4	3	5
Tarsimaculata	503	163	298	321
Tæniorhynchus	2	5	2	28
Culex spp	19	13	12	69
Total	530	185	315	423

The oil of creosote was the most effective repellant, whereas oil of sassafras seemed to attract *Anopheles*. Reduction was most marked with the culex spp. The men in the nets reported many instances of *Anophelenes* biting through the film of oil. There is a fruitful field of investigation still open, which should yield a substance with which we may attract thousands of mosquitos, and by the arrangement of traps, destroy these. Experiments on the Zone yielded nothing worth while so far.

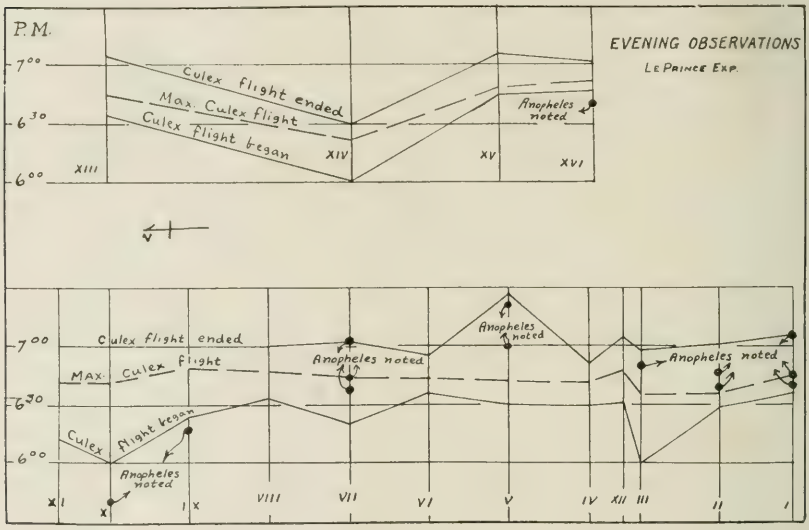
* The weight on the stomachs of the four birds killed was: 3.55, 3.5, 4.05 and 3.9 gms. They contained 49 large yellow ants, 30 pentatomids, 6 coleoptera and a mass of greatly disintegrated insect remains. In the esophagus were found five *Anopheles*, and in the mouth cavity of one bird were two more.

C. BEHAVIOR.

1. Flight.

a. By Direct Observation.

I. The Le Prince Observations: Mr. J. A. Le Prince detailed sixteen sanitary inspectors to report at Gatun on March 28th, 1913, to observe the evening and morning flights. A graphic representation of the positions taken and the facts obtained are given in the following two charts and map. A line of observers was strung on the peninsula and the island, parallel to the old French Canal, intercepting the line of flight of the mosquitos for more than a mile. Four additional observers were stationed along the railroad at and beyond New Gatun.

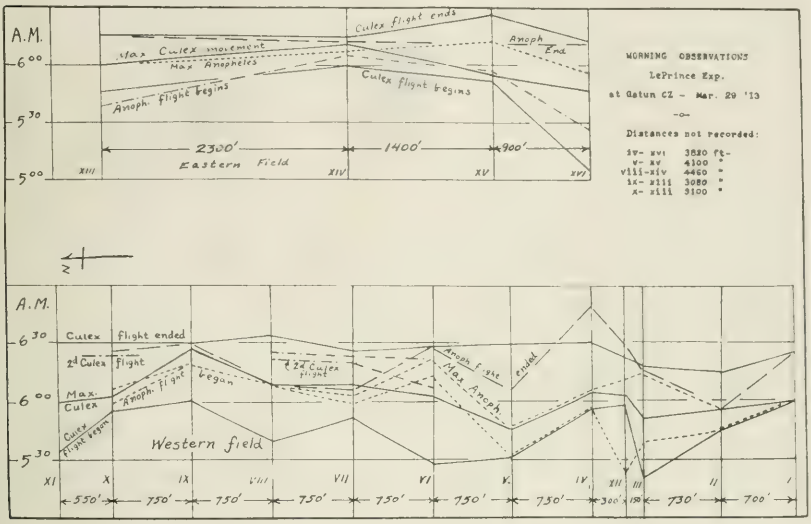


The instructions given were:

To observe (1) when the first culex appeared; (2) when the first *Anopheles* appeared; (3) when the culex were thickest; (4) when the *Anopheles* were thickest; (5) when the culex flight ceased; and (6) when the *Anopheles* flight ceased. The men were at their posts by 5:30 p. m. on the 28th and by 5:30 a. m. on the 29th, early enough not to miss anything.

The charts show a fairly even flight along the line parallel to the breeding place. The evening flight began at a little

after six p. m., its maximum at about 6:40 p. m., and was completed by about 7:00 p. m. The morning flights began at about 5:55 a. m., the maximum return Anophelene flight at about 6:05 a. m., ending at 6:25 a. m. The Anophelene return flight ended sooner than did that of *tæniorhynchus*. The morning flight is shorter in duration than the evening one, is marked with greater precision than the evening one and takes place higher above the ground than during the evening.

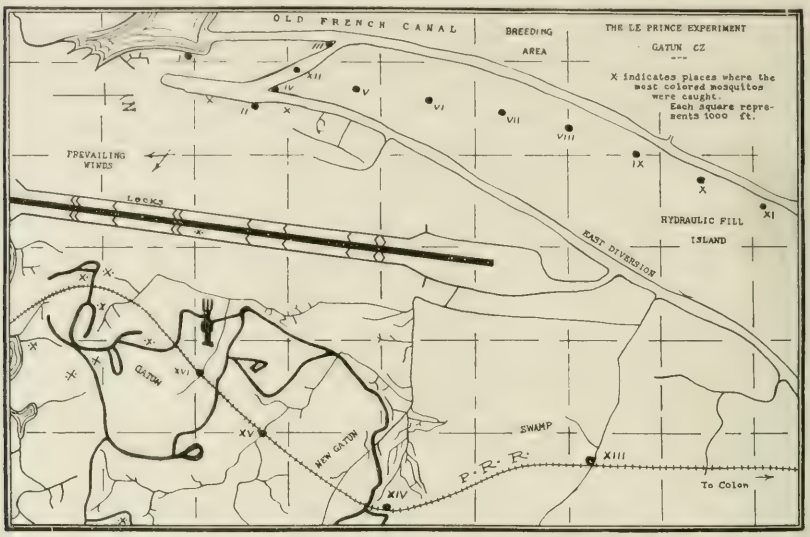


The important facts obtained during this extensive experiment were: 1, the finding of blood in mosquitos of the return flight; 2, finding of males in the return flight; and 3, the definite mechanical adjustment to light intensity. More such experiments as these of Mr. Le Prince are needed; they yield more useful data than a lot of learned speculations over a few ideas.

II. Observations from Boats: While mosquitos were being trapped at the swamp, two inspectors in a small row boat moved up and down the old French Canal, and by lying on the bottom so as to have the sky for a background, one of the men would observe the mosquitos as they flew past. This method was the first used to learn that a flight actually was taking place.

b. Demonstration of Flight.

I. The Staining and Recovery of Marked Mosquitos: In his previous paper (1913-a) the writer devoted considerable space to the need of collecting large quantities of larvæ and pupæ and how these should be treated to yield a maximum amount of adults. At that time this was the only way adult mosquitos could be had in sufficiently large numbers. At Gatun, as already stated, the conditions met with presented a great improvement over this tedious and often disappointing method.



The Anophelenes and *Aedes tæniorhynchus* were so prolific that several thousand could be trapped within an hour. The special advantages of this, aside from the saving of time and expense, were, (1), that the adults matured in their natural environment and not in breeding cages; (2) they were an integral part of the hordes which would begin that same night their hasty journey to Gatun; (3) they were caught the same late afternoon they were to be liberated and could be set free at the exact moment that the flight began. The only unnatural feature was the addition of the tiny speck of anilin dye, but this can hardly produce much inconvenience to the mosquito since the spray used was extremely fine. If the spray had been very coarse and prolonged, it is easy to see how the speck of dye would cause mechanical irritation.

Mosquito bar nets were stretched out at the breeding place, three sides of which were in close contact with the ground while the remaining side was raised about a foot or so from the ground. At about 5:00 p. m. a negro was placed inside of each net, with instructions to prevent as far as possible mosquitos biting him. Within an hour Mr. Negro shared his net with a thousand or more noisy mosquitos. More mosquitos entered nets when these were kept dark.

The noise made by these dens of voracious, unrestful culicids, their persistent, unceasing attacks, and the endurance of the willing and patient negroes, are things that can never be forgotten by those who had witnessed them.

After having the nets full of mosquitos, the next step was to spray them. The usual vaseline nebulizer was used, and a 1 : 100 solution of anilin dye in water sufficed to give enough color to the mosquito so as to be easily recognized if found later on. Spraying was facilitated by illumination from the outside. Two net-fulls of mosquitos were killed after sprayed and when tested showed a percentage of 98 positive. Within fifteen minutes after spraying the nets were inverted and the mosquitos allowed all the freedom they cared for. It was assumed that at the end of this period a sufficient number would be in condition to take part in the flight to Gatun.

At the same time similar nets were placed opposite the breeding place and elsewhere, with men, dog or hens as bait, and the mosquitos thus caught were examined for color. The results are given elsewhere. It is well to state here that due to the fan-like spreading of mosquitos from the breeding place, and the unusually well-screened and protected homes in Gatun, one cannot expect a large number of colored mosquitos in the houses. The proportion of colored mosquitos to non-stained ones is probably as high as one to three hundred.

The following table gives the time, numbers, etc. of the marked mosquitos liberated. The direction, velocity and trend of the wind at the time of liberation is also indicated. Attention is called to the fact that the winds at such hours were generally of high velocity—a fact which should not be forgotten when one reads so many accounts that mosquitos do not fly when the winds exceed four miles per hour. Such speculative deductions are the natural results of looking upon

a mosquito from the standpoint of man's level, rather than interpreting its behavior from a dipterous standpoint.

L. Liberated at Salt-marsh, 1913.

Date	Number	Liberated	Color	Wind		
				Dir.	Vel.	Trend
Jan. 21	3,000	9:00 p. m.	G. V.	N	14	decreasing
" 22	3,000	9:00 "	"	N	11	"
" 23	3,000	9:00 "	"	N-E	4	"
" 24	500	7:00 "	"	N	11	"
" 24	3,000	9:00 "	"	N	9	"
" 25	3,000	9:00 "	"	N	7	increasing
" 27	500	5:45 "	"	W	5	decreasing
" 27	4,000	8:00 "	E O	W	5	"
" 29	500	5:45 "	"	N	19	"
" 29	3,000	8:00 "	"	N	17	"
" 30	500	5:45 "	"	N	14	"
" 30	2,000	8:00 "	"	N	10	"
" 31	2,000	8:00 "	"	N	10	"
Feb. 1	2,000	8:00 "	"	N	6	"
" 3	2,000	8:00 "	"	W	5	"
" 4	2,000	7:45 "	"	N	13	"
" 5	2,000	7:45 "	"	N	16	"
" 11	1,200	6:15 "	"	N	12	"

Total liberated, 37,200. G. V. = Gentian violet; Eo = Eosine.

The above table shows that the actual ideal was not followed, i. e., all liberations should have been done before 6:00 p. m. However, it will be seen from the data of recovered mosquitos, that the first colored ones found were on the 24th of January, and the first lot of colored mosquitos liberated was on the 21st. But these did not participate in the flight to Gatun until the evening of the 22d. Therefore, they entered the buildings either that same evening or the next day. In future experimentations of this sort, marked mosquitos should be liberated just when the flight is to commence.

All mosquitos caught in Gatun and New Gatun and occasional lots from Spillway Camp, New Frijolles, Monte Lirio and Camp Purdum, were tested for color, the method used being the same as in the author's previous paper (1913-a). Collection of adults was accomplished in the following manner: (1) a daily search through all buildings, made by expert negroes equipped with a wide-mouth vial containing a cotton plug saturated with chloroform; (2) by means of the "C. H. Bath" mosquito trap attached to barracks; and (3) by placing mosquito

bar nets under buildings with negroes as bait. A table of recovered mosquitos is inserted here:

M. Colored Mosquitos Recovered at Gatun, C. Z.

Date	Place Captured	Species	Color	Distance from Breeding
Jan. 24	Hotel No. 1	1 *albimanus?*	G. V.	4,875′
" 24	Q. M. D. Storehouse	1 *albimanus*	"	1,500′
" 24	Cement shed	1 "	"	2,000′
" 24	Lock Chamber	1 indet.	"	3,000′
" 29	" "	1 *tarsimaculata*	E O	3,000′
" 31	" "	1 *albimanus*	G. V.	3,000′
Feb. 1	W. of Locks	1 *tarsimaculata*	E O	2,750′
" 5	Lock chamber	1 "	"	3,000′
" 7	" "	1 "	"	3,000′
" 7	unknown	1 "	"	.....
" 8	Camp 83	1 "	"	4,125′
" 8	Bldg. No. 1006	1 "	"	5,000′
" 8	Hotel No. 1	1 "	"	4,875′
" 11	Camp No. 5	2 "	"	5,125′
" 11	Hotel No. 1	1 "	"	4,875′
" 11	" "	1 culex spp.	"	4,875′
" 11	Commissary	1 *tarsimaculata*	G. V.	4,375′
" 11	Bldg. No. 226	2 "	E O	4,400′
" 11	Col. Sibert's	1 *albimanus*	"	6,200′
" 11	" "	4 *tarsimaculata*	"	6,200′
" 11	Camp Ashton	14 "	"	5,125′
" 12	Administration Bldg.	1 "	"	4,375′
" 13	" "	3 "	"	4,375′
" 18	Camp Ashton	2 "	"	5,125′

Total: *albimanus* 5; *tarsimaculata* 38; *culex* sp. 1; indet. 1.
45 recovered—6 G. V. and 39 Eo.

Attention is called to the finding of a violet colored mosquito on the 11th of February, fourteen days after this stain was last used. It is hoped an opportunity may present itself to some one to be able to stain no less than twenty thousand mosquitos one night, and two weeks or so later to stain a similar quantity, using a different stain each time. By careful recovery of mosquitos in the townsite and environs, one should get some idea as to the longevity of individuals.

The number of recovered mosquitos is not phenomenally large, but it is conclusive proof that the mosquitos seen flying from the marsh toward Gatun, actually entered that town. About sixty specimens were found which showed a very faint tinge of color when the testing solution was applied, but these were not considered; only specimens which showed a decided color reaction have been incorporated in the table. When

nets which had been used for staining mosquitos were to be used under buildings, a careful search was made to eliminate any dead, colored mosquitos which might be entangled in the meshes of the net; and if by any chance a hard-dried mosquito was found among a fresh lot, it was always discarded. In this way it is believed all chance errors were eliminated.

From Feb. 8 to 21, mosquito bar nets were placed at several sites, with negroes again as bait, assisted at times by dogs and hens. During these trials 173 colored mosquitos were recovered. Those near the marsh do not figure in the table of recovered mosquitos. The following table presents the data for twenty-four separate nets, during six days. The Anopheles take mostly to men, not merely because of larger surface area, but because these insects prefer human beings to dogs or hens.

N. Mosquitos Caught in Tents, 1913.

Locality	Feb.	Bait	A	T	TE	C	Total	Recovered
Hydraulic Fill.....	8	Hen	...	12	1	6	19	none
" "	8	Man	7	342	85	53	487	25 T-red; 3 C-red.
" "	8	"	14	641	41	76	772	21 T-red; 2 C-red; 1 T-blue
Peninsula.....	8	Hen	...	23	.....	9	32	1 C-red
"	8	Dog	...	5	4	9	18	none
"	8	Man	2	273	10	15	300	7 T-red; 2 C-red; 1 T-blue
Hydraulic Fill.....	10	Hen	1	108	11	10	130	3T-red;1C-red;1T-blue
" "	10	Man	6	585	35	60	686	27 T-red; 8 C-red; 4 Te-red
" "	10	"	9	578	12	20	619	22 T-red; 4 C-red; 1 Te-red
Peninsula.....	10	Hen	...	2	1	.....	3	none
"	10	Dog	...	9	1	2	12	1 T-red; 1 C-red
"	10	Man	...	51	3	3	57	2 T-red
Col. Sibert's.....	11	Man	1	7	46	8	62	1 A-red; 4 T-red
Hydraulic Fill.....	12	"	...	12	3	.....	15	none
" "	12	"	1	69	5	1	76	4 T-red; 1 C-red
" "	12	"	6	478	64	48	596	28 T-red; 3 C-red
Administration....	12	"	1	148	20	8	177	1 T-red
Col. Sibert's.....	12	"	...	2	4	2	8	none
" "	13	"	...	3	5	5	13	"
Administration....	13	"	3	427	1	6	437	3 T-red
Hydraulic Fill.....	14	"	8	258	27	201	493	Rain throughout,
" "	14	"	4	278	14	168	454	heavy at 7:00 p. m.
Peninsula.....	14	"	...	51	2	27	80	Note abundance of
Administration....	14	"	...	22	1	13	36	culex, mostly Mansonia titillans.

Time: 8th-11th, incl. from 5:00 to 8:00 p. m.; 12th, 5:00-6:15 p. m.; 12th Admin. bldg. and Sibert's, 6:00-8:00 p. m.; 15th, 5:00-7:00 p. m. A=*albimanus;* T=*tarsimaculata;* TE=*tæniorhynchus;* C= *Culex* spp.

About fifty per cent of the mosquitos in the nets having dogs or hens as bait, showed blood meals, but there is no way of telling whose blood it might be. In one net, a patient negro combatted no less than fifteen thousand sand-flies! Horseflies occasionally appeared in nets.

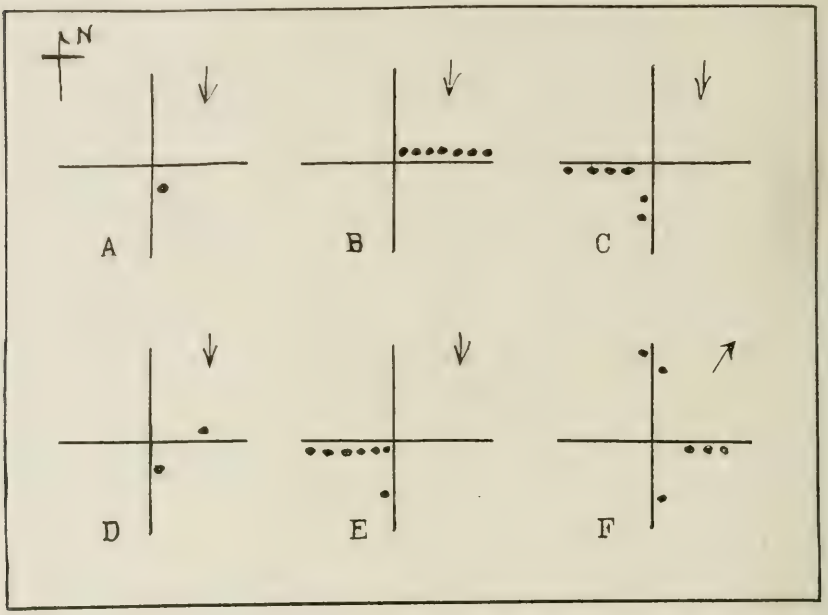
II. Quimby's Intercepting Planes: Mr. E. Frederic Quimby, a Division Sanitary Inspector, invented and had patented a contrivance by means of which he could detect the direction of flight of mosquitos. In principal his apparatus is much like that used in Massachusetts to trap young caterpillars carried by the wind. Haskell (1913) described the apparatus and the claims given to it by the inventor.

The scheme consists of a metal frame, holding four equal plates of glass, about a foot square each, at right angles to each other from a common vertical. The framework is mounted on a tripod which can be adjusted for height. The plates are smeared with a thin coating of transparent tanglefoot when in use.

The data of his tests are abstracted from his reports to the Chief Sanitary Inspector, and are graphically represented in the following plate. In every instance the instrument was set up at places where flight of mosquitos was known to be occurring. Thus far there is very little data on hand to validate the far-reaching conclusions made by the inventor, however, in making this statement the writer does not wish to convey the idea that the apparatus is no good. The principal is right, and all it needs is sufficient experimentation to perfect it. If several instruments were set up and worked without man's presence, the resulting catch would give valuable clues as to the direction of the breeding place from the town-site. Once the instrument is known to be fairly accurate, its use may become general in indicating those areas about a given town-site which require thorough sanitation, and the elimination from control of areas suspected as dangerous but which the experimental data shows relatively safe.

Referring now to the diagrams, the dots represent mosquitos actually caught on the sides nearest which these appear, and the arrows indicate the direction of the wind at the time. With a wind of 19 miles per hour at Mount Hope, "A," *one* mosquito was found as indicated, and the conclusion was at

once reached that the breeding place was to the south-east of the instrument. But that same evening, the same place and but an hour later, the wind increased to 25 miles per hour, and as is shown in "B," there were *seven* mosquitos caught on the north side of the east plane! If the conclusion based on the single specimen in "A" was correct, then the seven mosquitos under "B" indicate a breeding place to the north-east. The discrepancy is due to the fact that the inventor was close to his instrument, to the north-east of it, and mosquitos attracted



0- Quimby's Data.

to him were blown against the glass sides due to the high velocity of the wind at that time. Mr. Quimby states in his report that although mosquito activity was quite observable, it ceased entirely fifteen minutes after the wind increased to twenty-five miles per hour.

The remaining four charts refer to Gatun, and in this case the breeding place was definitely known to be to the west of the instrument. "C" and "E" are evening tests, while "D" and "F" are morning trials. In case "F" the wind was from

the south-west and therefore some irregularities occurred. In all cases the instrument was set about four feet from the ground. More mosquitos were caught during the evening flight. This is because the morning flight takes place higher in the air and the instrument was too low down to intercept it.

Inasmuch as the glass planes are a barrier to the wind, it is worth while to consider the fact when deductions are being made. Our results, covering several years of records, show more mosquitos caught in traps on the leeward of buildings than to windward. Examining Quimby's charts, a similar condition appears to exist. Cases "C" and "E" show more flies on the south side of the west plate. The mosquitos flew at a quarter to the wind, i. e., in a south-easterly direction. Therefore more mosquitos should have been found on the west side of the south plate and the north side of the west plate. The actual catch shows more mosquitos on the lee side, and therefore the breeding place should have been to the south-west and the mosquitos bound for the north-east, which of course was not so.

A few tests were made using copper screen instead of glass, but the tanglefoot produced a coppery odor and verdigris, the results proving negative. A cloth screen, larger in area, would probably yield better results. One instrument is not enough; as they are inexpensive, several dozen should be placed about a suspected area, much on the order that mines are planted across the paths of ocean traffic. And not the least in importance human beings must keep away from these screens as long as the mosquitos are active.

2. Mosquitos Harboring Under Buildings.

Since the houses in Gatun are so well screened, and cracks in the floors and walls stopped up, mosquitos enter with much difficulty, the majority of them gaining entrance as doors are opened and closed. It was hardly thought possible that all the mosquitos that flew to Gatun one evening, returned the next morning. Therefore mosquito bar nets were spread under four buildings and a negro placed under each one, equipped with a chloroform-filled killing tube. His instructions were not to impede the entrance of mosquitos and to catch all that entered.

The data is summarized in the following table. (Alb—*albimanus;* Tar—*tarsimaculata;* Tae—*tæniorhynchus;* Tit—*Mansonia titillans*).

P. Mosquitos Caught Under Buildings, Gatun.

DAYTIME CATCH.

Number of Buildings	Days	Periods	Anophelinæ		Non-Anophelinæ			Total
			Alb	Tar	Tae	Tit	Others	
4	28	108*	37	11,604	3,496	258	766	16,161
Percent of total			0.4	71.7	21.6	1.6	4.7	
Average per period			0.3	111.8	33.7	2.5	7.4	155.7
Males taken			1	265	No count kept			

*Four periods lost through invasion of ants.

Thus it is seen that at least 112 *Anopheles* lingered under each building. Assuming that there are only one hundred buildings in Gatun, then each day there harbored under these houses no less than ten thousand malarial mosquitos. And considering New Gatun, open and unscreened, one must use his imagination freely to arrive at some suitable conclusion. Had it not been for the thorough treatment given in the past to malaria sufferers, and had not a daily search been made in all buildings for mosquitos, then surely the malarial rate would have leapt upward, probably paralyzing for some time the work at this important section.

All males caught were examined microscopically for the presence of blood in the digestive tract, but all tests proved negative. In nearly all females of *Anopheles* blood was found but since only a very few showed a very recent meal (upon the negro bait), it was reasonable to suppose that the males were taken the night previous. Chironomidæ appeared in great numbers at times, and from April 8th to 12th, out numbered by far the total mosquito catch.

The hourly distribution of mosquitos caught during the night is given in the following two tables. The actual paucity of data permits of no accurate deductions. However, it is significant to note the conspicuous catch of 233 *tarsimaculata* under

Colonel Sibert's home, from five to six a. m., the time of maximum activity in mosquito world. The writer recalls that mosquitos were always most active during dusk and dawn, when he was making all-night observations in tents.

Q. At Gatun Locks, February 18 and 19.

	Hour Ending at												
	P. M.						A. M.						
	7	8	9	10	11	12	1	2	3	4	5	6	Total
albimanus	5	1	..	6	4	3	3	1	..	1	..	2	26
tarsimaculata	40	18	41	95	88	47	51	22	19	14	12	34	481
tæniorhynchus	..	..	..	..	..	..	..	..	..	..	..	2	2
culex spp	..	1	..	..	1	..	1	..	..	..	2	..	5
M. titillans	3	2	2	4	1	..	..	..	..	1	1	2	16
albimanus	1	2	1	..	..	..	..	..	..	..	1	1	6
tarsimaculata	..	30	27	13	7	8	12	..	6	8	21	14	142
tæniorhynchus	..	..	..	..	..	..	..	..	..	..	..	1	1
culex spp	..	2	2	1	1	..	1	..	..	..	..	..	7
M. titillans	..	6	9	6	4	1	1	..	1	1	2	2	33

R. At Col. Sibert's Home, February 15, 18 and 19.

	Hour Ending At												
	P. M.						A. M.						
	7	8	9	10	11	12	1	2	3	4	5	6	Total
albimanus	..	..	..	..	3	1	..	1	2	2	..	..	9
tarsimaculata	1	2	2	1	10	11	8	13	5	4	..	2	60
tæniorhynchus	..	..	1	..	..	..	..	..	..	..	..	..	1
culex spp	..	2	..	4	..	1	..	4	1	2	..	..	14
M. titillans	4	29	4	18	48	38	21	24	26	8	..	5	222
albimanus	..	1	..	..	3	2	..	..	2	..	1	12	20
tarsimaculata	2	4	7	3	14	7	1	1	3	..	17	233	292
tæniorhynchus	..	2	..	..	..	1	..	..	..	..	..	2	5
culex spp	1	1	2	..	1	..	1	..	1	1	1	4	13
M. titillans	..	11	7	9	1	14	1	2	4	2	2	4	57
albimanus	..	3	..	..	..	..	..	1	4	..	1	..	9
tarsimaculata	1	78	1	13	2	11	5	32	36	3	22	4	208
tæniorhynchus	..	..	..	..	..	1	..	1	..	..	..	..	2
culex spp	..	1	..	..	2	..	..	..	2	1	..	2	8
M. titillans	3	12	17	2	4	17	2	4	3	14	..	9	87

The data for the mosquito catch under buildings during the daytime shows that 33.42% of the total catch is made from 6:30 a. m. to 8:00 a. m. Several tests were made to note whether the color of the ground had any effect on numbers. White and black ground were used, and the day's catch of the two was taken as 100%. Of this white ground yielded 39.33% and black ground 60.67%. The question of color of barracks, color of interior, color under houses, location of doors, of windows, etc., have greater importance in the reduction of the numbers of mosquitos than is at first believable, and more definite studies along these lines are needed.

3. Securing Food and Eating.

To describe adequately the voracity of the female *tarsimaculata* or *tæniorhynchus* is to attempt something bordering on the impossible. For indeed language falls short when one tries to narrate what he has witnessed. No matter the time of the day or night, just as soon as one left the boat and entered the jungle of the marsh, hundreds of hungry mosquitos flew forth to warmly welcome the intruder. And if the intruder had come just as dusk appeared, the welcome he received was simply tormenting. Despite all efforts to drive these mosquitos away, he would carry with him no few bites. Laboratory tests showed these two species ready to suck blood when but eighteen hours of age.

There is preference shown to leather leggings and khaki cloth. Since these have a definite odor, probably odor as well as color are the attractive features. Never was there noted an indisposition to eat, and satiation bordered always on gluttony. The proboscis, aided by the palpi, probed assiduously khaki, leather, etc., very often succeeding through cloth. When the proboscis enters the flesh, very little pain is felt, .. e., not nearly so much as some Culex can produce. Frequently the beak is withdrawn and reinserted with increased vigor. Not infrequently females would so gorge themselves with blood that the hind feet could no longer sustain the weight. The normal position of the mosquito is with the body at forty-five degrees, but when feeding, this position of the mosquito on the person is not of such great importance. Mosquitos were seen hanging from the under side of the arm. When the

abdomen hangs vertically, the mosquito cannot gorge itself as much as when in other positions. By placing a paper prop under the abdomen, so as to sustain the weight of the body, the mosquito was able to gorge itself to its utter satisfaction.

Leather leggings were rapidly lined with mosquitos. By covering one legging with light colored paper, very few mosquitos alighted thereon. When black paper was used, more mosquitos were attracted. When the leggings were thrown away, mosquitos still alighted on them in hopes of finding flesh. After sprinting, so as to promote perspiration, and then covering the bare feet with white paper, mosquitos alighted in numbers on the paper, but when black paper was used, nearly triple the number of mosquitos appeared. The sox of negros when thrown into the bush were found to be covered with mosquitos. All this tends to show a similarity of odor between khaki, leather and flesh, and that darker colors are preferred to light ones.

At the breeding place, and later confirmed at the laboratory, interesting feeding reflexes were observed. The *Anopheles* and *Aedes* were so voracious that their persistence in getting their proboscis into flesh was remarkable. Once inserted, the mosquito became almost insensible to pain or to intense light or heat.

Hitting the mosquito with a pencil was not enough to cause it to fly away. Bright sunlight focused upon it with a mirror was effective only after the stomach was well gorged with blood, and quite likely the mosquito left for other reasons. When heat rays were applied to the abdomen by means of a lens, a mosquito quickly flew away. To continue and learn how far mutilation could proceed, the abdomens of eight mosquitos were quickly severed with fine dissecting scissors. In three cases the mosquitos kept on sucking blood despite the injury. The remaining five flew away, and of these four returned for another suck of blood. This sort of behavior savors of the fictitious, however, it proves how deeply implanted may be a stimulus such as hunger, and how slavishly it is obeyed.

When allowed to feed unmolested, and holding the hand toward the sun, the writer was able to observe the passage of a liquid through the proboscis into the flesh, presumably salivary fluid, and following this, he could see the blood being sucked

up. Then came a strange performance. From the anal opening squirted out small droplets of clear liquid, slightly alkaline, and which the writer believes may be a part of the salivary fluids which entered the wound. These droplets soon assume a light reddish tinge which gradually increases in depth until blood-red. By allowing these droplets to fall upon a cover glass and examining these microscopically, it was learned that they actually were human blood. Darling (1912, p-14), refers to a similar reflex. In 1902, Schuffner noted the same performance, and probably further investigation will show that the salivary fluid which enters the blood is not for the purpose of preventing coagulation, but rather to flush out and prepare the alimentary tract for the blood meal. Laboratory experiments made by the writer indicate that after an *Anopheles* has gorged itself with blood, it does not feed again until the pellet of waste had been expelled.

The insect gorges itself with blood until often it increases the weight by two times. Darling (1912, p. 14), gives the weights of several *albimanus*, his data being:

Bred in laboratory, 24 hours old, midgut empty	0.0008 gms.
Bred in laboratory, moderate blood feeding	0.0016 gms.
Caught in labor cars, some blood in midgut, half-developed ova	0.0019 gms.
Caught in labor cars, much blood and early development of ova	0.0035 gms.
Caught in labor barracks, blood in midgut, no development of ova	0.0018 gms.
Caught in labor barracks, blood in midgut, no development of ova	0.0021 gms.

4. Regarding the Behavior of Anopheles.

(General)

Behavior is adaptation to the environment. The focus of the environment and the behavior of the organism is the physiological state of the animal—a dynamic condition. External stimuli, if they are to cause change, must enter and alter the existant physiological state. If the stimulus does not produce movement, it may produce a new change in the physiological state. But a given stimulus which produced a certain change, may not produce the same change always. For example, hunger may modify a response.

High humidity, winds, salinity of water, etc., may alter the environment and by so doing, create a condition of stress. The result of all change is relative unstable equilibrium, and a tendency exists to relieve this stress and approach equilibrium.

This often means collision with unfavorable conditions, and the animal moves away, a negative response. If it copes repeatedly with such new conditions, it must become attuned to these conditions. If it cannot do so, it must give in to that form or association of forms that can become attuned. Successful attunement results in greater hardiness and ability to cope well with other new conditions.

The gradual domestication of *A. albimanus* is the result of just such collision. The condition today is not the result of some sudden change, but represents gradual attunement. And probably the fact that the breeding at the swamp, which at first was largely *albimanus* s. s., later changing almost entirely to *tarsimaculata*, which presented conditions of extreme salinity and a difference in specific gravity, is another demonstration of just such collisions. There probably exists among the total of any species groups of individuals having one or more strains in them which permit them to endure conditions of greater stress. And so we may readily suppose that the transition from true *albimanus* to the racial variety *tarsimaculata* is the result of just such existant strains.

(Specific)

Regarding flight, two definite facts appear; (1), there is a marked flight during dusk and dawn, and (2), this movement stops with almost mechanical precision when there is too much or too little light. The evening flight was always low and deliberate. That of the morning was characterized by haste and took place higher in the air. The factor which determines the height at which the flight occurs is probably humidity. The duration of the flight depends upon the duration of the zone of light of that degree of intensity to which the mosquitos are accustomed to.

Several laboratory experiments were made to determine the reactions of these mosquitos to light. A large number of *A. tarsimaculata* were confined in a large glass jar which was covered with several thicknesses of black paper. The top was covered with a board in which was drilled a one-half inch hole. Upon this cover was placed a lamp chimney with the top of it covered with gauze.

(a) The apparatus was set up in front of a window. No mosquitos entered the upper chimney until the sun was low and dusk appeared. Then they appeared in large numbers and congregated about the gauze top. At dawn they also appeared on this gauze top, and they attempted to get away.

(b) Repeated, excepting that the chimney was covered with light paper so as to produce within a semi-darkness not far from that of dusk. Apparatus set up in front of window in bright sunlight. Mosquitos entered the top chimney irrespective of time of day. By removing the paper cover, they scrambled to get into the lower dark cage.

(c) Modified by removing the gauze top of the chimney and by adding to this chimney another one. The top chimney and the bottom one were kept dark within. The middle chimney was kept in semi-darkness. Mosquitos entered middle chimney. The wrapping of this was then taken off, thus admitting bright light. The mosquitos flew into the upper dark chimney.

These crude experiments and the field observations show that these mosquitos are attuned to that range of light intensity which occurs during dusk and dawn. When this optimum intensity is increased, the mosquitos become negatively phototropic.

When hungry, mosquitos are positively chemotropic and this stimulus may alter the effects of intense light and lessen greatly those of heat.

During flight the phototropic response is the dominant one. But observations seem to indicate a positive anemotropism, i. e., an orientation with reference to the direction of the wind. The mosquitos seem to fly at a quarter to the wind. The flight at Gatun was more than one mile and was made in one continuous flight. The observations of Celli in Italy that Anopheles do not fly further than from two to three hundred and fifty meters, indicates a different set of conditions existing in Italy than in Panama. One of the very first things to do in malaria sanitation is to learn the species involved, which of these transmit malaria, and then scour the literature for all facts regarding the behavior of the disease-carriers. Then studies should be carried on to learn something definite about the flight factors, and these studies cannot be of value unless the entire environ-

ment is considered. Such information may indicate the particular places which require strict anti-malarial measures, and may eliminate from thorough treatment certain suspected areas, thus reducing greatly the cost of sanitation.

SUMMARY.

(1). The study of the behavior of mosquitos has important value to the sanitary inspector in that this study gives him important clues as to the measures to adopt, which areas to control, and where best to locate temporary or permanent camps.

(2). The factors of wind, temperature and humidity are the most important in the adult mosquito environment. The winds at Gatun are relatively high, but they die down considerably as dusk appears.

(3). The salinity of the water equalled that of the ocean, but did not inhibit breeding. Ordinarily, the water of the salt-marsh is greatly evaporated as the dry season advances, and its salt content so increased that mosquito larvæ cannot live therein. However, there was an increase of rains the latter part of 1912 which so diluted the water of that marsh that the subsequent evaporation did not increase the salt content beyond the critical point.

(4). The life cycle of *Anopheles tarsimaculata* was found to be from seven to nine days.

(5). Direct observations from boats and on land showed a distinct flight of hordes of *tarsimaculata* and *tæniorhynchus* toward Gatun, beginning at dusk, and lasting about thirty to forty-five minutes. There was a return flight from Gatun to the breeding place beginning at early dawn and lasting until objects could be easily discerned, about thirty minutes duration. This return flight takes place higher in the air, and is characterized by haste.

(6). The flight to Gatun was experimentally proved by liberating marked mosquitos at the swamp and later recovering them at Gatun.

(7). Copulation probably takes place on the return flight. More males were found near the marsh during the return flight than during the evening flight.

(8). Mosquitos were found to harbor during the daytime under buildings in Gatun, a low estimate placing the *tarsimaculata* population under buildings at ten thousand per day.

(9). The *tarsimaculata* and *tæniorhynchus* exhibited extreme voracity, and would continue to suck blood although placed in direct sunlight. While feeding, the abdomen of several adults was snipped off without causing apparent pain or total cessation of activities.

(10). These mosquitos would alight on a coat hanging on a tree, or in a cast-away pair of leggings. Odor probably is the main impulse, though preference to dark colors is shown. The mosquitos actually were trying to sink their proboscis into the leather and cloth.

(11). Both species, when allowed to feed unmolested, ejected from their anal openings droplets of clear fluid which later became tinged with reddish, finally resulting in pure blood.

(12). Behavior is defined as adaptation to the environment. Successful attunement to the environment infers repeated collisions with unfavorable conditions and successful coping with these conditions. The apparent domestication of *albimanus* appears to be the result of such attunement brought about through combatting successfully new and adverse conditions.

(13). During flight the phototropic response is dominant. At the breeding place this tropism is often over-ruled by that of hunger. When in flight, the mosquitos seem to adjust themselves with respect to the wind so as to gain the greatest benefit therefrom.

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Ancon, Canal Zone, May 30, 1915.

SOME NEW CHALCIDOID HYMENOPTERA FROM NORTH AND SOUTH AMERICA.

By A. A. Girault,
Bureau of Entomology, U. S. Dept. Agriculture.

1. **Pseudleptomastix** new genus of the Ectromini.

Female: In my table to the earth's genera runs to *Paraleptomastix* Girault but the form is not robust, the head not lenticular, the face deeply inflexed, the scrobes joined above, the stigmal vein subequal to the marginal which is about two and one-half times longer than wide, yet distinctly somewhat shorter than the postmarginal. The costal cell is extremely narrow, practically obsolete for its greater length. Hind legs normal. Frons broad, sloping, the cheeks nearly as long as the eyes. The two teeth of the small mandibles acute but unequal in length. With the general habitus of *Ooencyrtus*. Hind tibial spur very minute.

(1) **Pseudleptomastix squammulatus** new species. Female. Genotype. *Length*, 1.00 mm.

Dark metallic green, the thorax purple, the abdomen black or nearly, the fore wing slightly dusky throughout, its venation dusky yellow. Antennæ black but the club, apex of scape and of pedicel rather broadly, yellowish white. Mandibles reddish. Legs yellowish white except caudal coxæ and femora to tip, a broad dusky band around middle femora near base and a spot above at apex. Head coarsely scaly, the thorax dorsad rather finely so, the triangular scutellum somewhat finer and denser than the scutum; abdomen across base densely scaly. Scutellum not reaching base of the abdomen, the axillæ a little separated. Abdomen about as long as the thorax, pointed. Pronotum arcuate, linear; scutum wider than long. Proximal joint of the hind tarsus much the longest. Pubescence on scutum white, not dense, short. Tegulæ large, white. Pedicel twice longer than wide at apex, longer than any of the funicle joints which are subequal, each nearly twice longer than wide, the club slightly wider than the funicle and about half its length, each of its joints a little shorter than one of the funicle joints. Oblique hairless line closed caudad, with many lines of cilia proximad of it. Fore wings normal, neither narrow nor especially broad, rounded at apex.

Described from two females labelled "State Insectary, California, 675" Fresno, California, from mealy bugs on grape.

Type: Catalogue *No. 19335*, U. S. N. M., one female on a tag and a slide with the appendages.

2. **Pheidoloxenus wheeleri** new genus and species of the Encyrtini.

Female: Head quadrate, apparently lenticular, the antennæ inserted near the mouth, 10-jointed with one very short ring-joint, the club solid; scape strongly compressed, widening strongly distad, the pedicel normal, the funicle compressed, its joints not annular but much wider than long, widening distad; club two-thirds the length of the funicle; pedicel slightly longer than wide at apex. Middle tooth of mandible obtuse, much the largest, the other two subequal and small. Maxillary palpi 4—, the labial 3— jointed. Wings mere scales. Legs simple, the middle tibial spur stout, the hind tibial spur single. Propodeum noncarinate. Abdomen with a short petiole, stout, oval, the ovipositor inserted at about distal fourth. Pronotum large, wider than long, distinctly longer than the scutum or scutellum, the axillæ separated a short distance.

Length, 1.85 mm.

Yellow brown, the club black; the body washed with metallic purple except the scape, funicle, pedicel and four proximal joints of the tarsi. Fore wings purple. Body scaly and with numerous minute pin punctures. Funicle 1 less than a third of the width of 6 but nearly as long, less than half the length of the pedicel. Fore wings nearly twice longer than wide at apex. Distal joint of maxillary palpus nearly as long as the other three combined.

From one female on a slide in the U. S. N. M., "From nest of Pheidole. *Pheidoloxenus wheeleri* Ashm."

Type: Catalogue *No. 19336*, U. S. N. M., the foregoing female on a slide.

This genus was partly described by Ashmead as an Asaphine Pteromalid. According to J. C. Crawford, Wheeler has already figured the species.

3. **Eunotus americanus** new species.

Female: Length, very variable, average about 1.20 mm.

Black, the head very dark green, the abdomen shining. Wings subhyaline, the venation pale yellow, the legs and antennæ reddish yellow, the coxæ subconcolorous, also the pedicel. Head densely finely scaly, nearly scaly punctate, the thorax a little more densely so. Scutellum a half longer than the scutum. Propodeum short, areolate or foveolate, the areas not large. Abdomen nonsegmented, glabrous. Postmarginal and stigmal veins subequal. Hind tibial spurs double, much unequal. Antennæ as in the figure of *acutus* Kurjumov but the second articulation of the club is absent, the club thus but 2-jointed; insertion on the clypeus or nearly. Lateral ocelli not touching the eye margin.

The male is the same but the antennal club is 3-jointed, the flagellum filiform and about as figured for *acutus*. Male mandibles bidentate, both teeth acute and equal.

Described from one male, two females on tags "Portland, Maine, 8–2–04. E. M. Patch. Bred from *Eriopeltis festucae.*"

Type: Catalogue *No. 19337*, U. S. N. M., a female on a tag, two female hind legs and an antenna, one male head on a slide.

4. **Blastothrix longipennis** Howard.

This species is very close to *B. sericeus* Dalman (Mayr.), but differs in having funicles 1–2 shorter, a little longer than wide and shorter than the pedicel while funicles 1–2 in the European species are each distinctly longer than wide and subequal to the pedicel. The scapes are greatly dilated, the mandibles *bidentate* as in *Anagyrus*, the marginal vein twice longer than wide, the stigmal and post marginal subequal, each a little longer than the marginal. The pubescence is normal, not scale-like. The genus runs very near to *Paratetralophidea* Girault, which has the marginal vein longer than the stigmal or postmarginal.

5. **Homalotylus obscurus californicus** new variety.

Female: Length, 1.50 mm.

Runs close to *obscurus obscurus* Howard but differs in that the head is all blue black except the antennal insertions and the mouth which are orange yellow; the entire body is blue black except the mesopleurum which is reddish marked slightly with metallic, wholly metallic distad and the middle tarsi which are white and the middle femora which are reddish yellow; also funicle 1 is only slightly longer than wide. Frons narrow; head lenticular; mandibles 3-dentate. Hind tibial spur distinct. Club obliquely truncate from base to tip, its segmentation very indistinct. Scutum with pubescence as in *Blastothrix*. Axillæ with a slight carina between them. Body very densely, finely scaly. Tegulæ brown yellow at proximal half or nearly, the rest black. The cross stripe of the fore wing is complete, fainter caudad and intersected by a narrow, transverse hyaline streak near caudal margin. Head with numerous scattered punctures. Marginal vein punctiform, the postmarginal and stigmal veins rather long, the former somewhat shorter than the stigmal, about five times longer than the marginal.

From four females labelled "Whittier, Calif., Jl. 12, 1912. Ex. *Cheilomenes sexmaculatus*. P. H. Timberlake, Coll. 14627 B."

Type: Catalogue *No. 19338*, one female on a tag and a slide with head, fore wing and a hind leg (plus paratype antennæ). Three paratype females on tags.

6. **Parataneostigma** new genus Taneostigmini.

Female: In Ashmead's table runs to *Eutrichosoma* but the body is practically naked. Differs from all the genera of the tribe in that the obliqued (meso-caudal) parapsidal furrows distinctly are incomplete but run toward each other to about the middle of the scutum (running nearly transversely) yet widely separated from each other at their mesal tips. Antennal club obliquely truncate from near the base, solid, the antennæ 9-jointed, inserted on the clypeus, the scrobes forming a long semi-circle; face inflexed. Genal suture subobsolete. Frons moderately narrow. Venation not quite reaching the costal margin, the marginal vein obsolete, the postmarginal distinct but very short, the stigmal long and slender. Axillæ very broadly joined, a carina between them.

(1). **Parataneostigma nigriaxillæ** new species.

Female: Length, 2.00 mm.

Bright canary yellow, the wings hyaline with the exception of a fuscous blotch from the stigmal vein, the latter fuscous, the venation yellow. The following jet markings. Face of pronotum except marginally, a stripe across above center of occiput, the axillæ, the propodeum, the abdomen except broadly at base, the caudal knees very narrowly, a cinctus just below them and a broad one farther distad, near apex of caudal tibia. Funicle and club dusky. Lateral margin of propodeum with a line of silvery pubescence. Body finely reticulated. Caudal half of pronotum blackish, the cephalic margin of this half with a regular cross-row of short, flattened, grayish setæ which are appressed. Scutellum embrowned just before tip. Lateral ocelli barely separated from the eyes. Pedicel twice longer than wide at apex; funicle joints all somewhat wider than long, widening distad.

Described from one female labelled "Mitla, Mexico, L. O. Howard."

Type: Catalogue *No. 19339*, U. S. N. M., the specimen on a tag, the antennæ and wings on a slide.

7. **Ceratoneura petiolata** Ashmead.

One female, San Rafael, Jicoltepec, Mexico. The antennæ bear four ring-joints, the fourth very large yet wider than long, colored and clothed like the funicle; club subsolid. No lateral carina on the propodeum. The oblique striae on the face are ventrad of the rather high antennal insertion. The three funicle joints are subequal, all somewhat longer than wide (a third longer or a little more); petiole distinct, not long.

8. **Ceraptrocerus** Westwood and **Habrolepis** Foerster.

The head in the first genus is not oblong, but short, the face much inflexed, the frons subprominent. The ovipositor is more or less exserted. *Habrolepis* differs in having the flagellum usual, the funicle joints only a little wider than long, but the scape is as in *Ceraptrocerus*. There appears to be no special armature on the scutellum. The mandibles in *H. zetterstedtii* are *quadridentate*, the two inner teeth weaker than the other two. Of this species, a female reared from *Lepidosaphes ulmi*, Manchester, Eng., A. D. Imms.

9. **Anagrus armatus** Ashmead **nigriceps** new variety.

Female: Like *armatus nigriventris* but the head is dusky black as in *Anagrus giraulti* Crawford (which is otherwise typical *armatus nigriventris*) but this new variety differs in antennal structure thus—funicle 3 is distinctly shorter than 2 or 4 which are subequal, each very slightly longer than 5 or 6. In typical *armatus* and in *giraulti*, funicle 3 is slightly longer than 4 and slightly shorter than 2.

This variety does not differ greatly from *Anagrus flaveolus* Waterhouse (specimens so labelled in the U. S. N. M.), excepting much in coloration, the fore wings broader and funicle 2 is hardly longer than funicle 6.

Described from two females on a slide, reared from eggs of *Empoasca rosæ*, Corvallis, Oregon, H. F. Wilson.

Types: Catalogue *No. 19340*, U. S. N. M., the above specimens.

10. **Camptoptera** Fœrster with 10-jointed Antennæ.

The following species have a ring-like joint in the antennæ: *C. gregi*, *C. pulla* and *C. saintpierri;* this joint is between joints 1 and 2 of the funicle. The antennæ are therefore 10-jointed. These ring-joints are distinct and the reason they have been overlooked heretofore is due partly to the use and custom, preconceived ideas and so on. Females only examined.

11. **Bothriothorax flaviscapus** new species.

Female: Length, 2.00 mm.

In Howard's table of species runs to *peculiaris*, but differs in having the scape all reddish yellow. Of the usual dark metallic green color, the axillæ separated by a short carina. Distal third of scutellum shining (yet finely scaly) together

with a narrow median line from this to the base of the scutellum. Scape above at tip slightly, tips of caudal tibiæ, bases of caudal femora and most of middle femora nearly to tip, dark, submetallic. Rest of legs yellow brown except the front femur nearly to tip, which is dark, submetallic. Funicle 1 subquadrate, the rest wider and wider than long. Club 3 jointed, obliquely truncate from near base of joint 2. Venation pale yellow, the wings hyaline, the marginal and postmarginal veins punctiform, the stigmal long. Head lenticular. Mandibles tridentate, yet like those of *Anagyrus*, except that the dorso-lateral apex of the truncate second tooth is subacute and projects beyond the rest; and a sinus is between the two teeth at base. Cheeks not so long as the eyes. Scape slightly foliaceous at tip. Funicle 6 distinctly wider than long, the pedicel longer than any funicle joint. Hind tibial spurs double, very unequal. Otherwise agrees with the description of *peculiaris*.

The male is about as in the female, but the antennæ are all yellow-brown, filiform, the club solid, the funicle joints hairy and much longer than the pedicel, which is barely longer than thick, each joint about half longer than thick and excised toward tip, a little. Club a little longer than the scape.

Described from two males, six females in the U. S. N. M., "From syrphid pupa. Ashmead." U. S. A.

Types: Catalogue *No. 19341*, U. S. N. M., a male and female on a tag; remaining specimens on three tags are paratypes, same number.

12. **Taneostigmine Genera.**

Taneostigmodes differs notably from *Taneostigma* in that the stigmal vein is normal, i. e., not straight and nearly perpendicular. *Eutrichosoma* has a much shorter marginal vein and the parapsidal furrows do not meet at all distad, yet at the scutellum not so much separated as usual. Also the postmarginal vein is absent. The scape is slender, the antennæ cylindrical, 13-jointed with one ring-joint, the club 3-jointed. The antennæ in the other two genera bear two ring-joints and are 13-jointed. *Taneostigmodes* occurs in Australia but other Australian species in the group represent a half dozen peculiar genera.

13. **Habrocytus rosæ** Ashmead.

Several specimens of both sexes, Brooklyn, N. Y., February 9, 1913, from rose, A. S. Berquist, Chtn. No. 970. The cross-furrow on the scutellum in this genus is really an obtuse cross-carina.

14. **Entedononecremnus** new genus of the eulophid Hemiptarsenini.

Female: With the form and sculpture of the Entedoninæ but there is distinctly no break in the submarginal vein, the abdomen is sessile and the stigmal vein of normal length. Head somewhat wider than long, the antennæ inserted somewhat below the middle of the face, 8-jointed with two large ring-joints, the club 3-jointed, large, conic-ovate, with a distinct, long terminal spine, the club longer than the rest of the flagellum, the funicle joint quadrate, shorter than the pedicel, large. Genal suture present. Pronotum not visible. Scutum large, the obtuse parapsidal furrows only cephalad; scutellum simple, without regularly placed bristles. Axillæ advanced half way cephalad of the scutellum. Propodeum short, somewhat longer laterad, with a median carina and an oblique (latero-caudad) lateral one. Scutellum projecting over the propodeum, the postscutellum absent. Abdomen flat from dorsad, nearly round, the ovipositor not extruded, segment 2 longest, occupying a little over a fourth of the surface. Marginal vein distinctly shorter than the submarginal, the postmarginal subobsolete, the stigmal long, a third or more the length of the marginal, its knob linear. Hind tibial spur short and stout. Mandibles small, bidentate. Male the same but the club more slender.

(1) **Entedononecremnus unicus** new species. Genotype.

Female: Length, 1.20 mm. Short, stout.

Dark metallic blue green, the head and scutum purplish to coppery, the abdomen distad of segment 2 nearly black. Wings hyaline, the venation dusky yellow. Antennæ except the blackened club, cephalic and middle femora and tibiæ (except proximo-dorsal third of tibiæ and dorsal femora), reddish brown. Head and thorax punctate, the propodeum and segment 2 of abdomen subglabrous; rest of abdomen densely scaly. Lateral ocelli distant from the eyes. Hind tibiæ at apex beneath reddish brown. Tarsi white, the large last joint black. Pedicel somewhat longer than wide at apex; funicle at apex with a short petiole. Club 3 forming half that region, 1 and 2 much wider than long. Scutum and scutellum with sparse pubescence which is moderately long.

Described from one male, three females reared from *Aleurochiton* species, March, 1915, near Georgetown, Demerara, British Guiana, G. E. Bodkin.

Types: Catalogue *No. 19342*, U. S. N. M., 1 ♂, 3 ♀s on a tag plus a slide with ♂ and ♀ head, female hind tibia and fore wing.

NEW CHALCIDOID HYMENOPTERA.

By A. A. Girault,
Bureau of Entomology, U. S. Dept. Agriculture.

1. **Mirzagrammosoma** new genus of the Elachertini.

Female: With the habitus of *Zagrammosoma* Ashmead but the scutellum without grooves, the vertex more elevated, elevated nearly for the length of the eyes which thus appear to be in the middle of the side of the head, the cheeks a little longer than the eyes. Antennæ inserted about in the middle of the face, the scape compressed (about three times longer than wide at apex), also the funicle and club, as in the named genus but there are two short ring-joints. Mandibles 6—dentate. Submarginal vein distinctly broken in *regularity*, distinctly longer than the marginal, the postmarginal two-thirds its length. Club not very distinctly jointed. Hind tibial spur distinct, not large. Parapsidal furrows rather long, meeting the small axillæ which are nearly entirely cephalad of the scutellum. Pronotum conical, as long as the scutellum which is about two-thirds the length of the scutum. Propodeum a little shorter than the scutellum, with a delicate median carina and no others, the spiracle very minute. Abdomen sessile, as long as the thorax, conical. Cephalic coxæ elongate. Elachertine in appearance.

(1) **Mirzagrammosoma lineaticeps** new species.

Female: Length, 2.05 mm., slender.

Purplish black, the legs except the hind femora and tibiæ (except the latter at tips) and head pale yellow (middle and hind coxæ not seen); a narrow pale yellow line down two-thirds of the pronotum from cephalic margin, latero-dorsad and ventro-laterad (total of four lines). Meson of proventer caudad broadly golden yellow. A broad black line down middle of the face from vertex to clypeus and another across the vertex and down each side to the eyes and then from the ventral ends of the latter to the end of the head across the cheek. Mandibles pale yellow, reddish at tip. A pale golden line up the lateral margin of the axilla and along the scutum some little distance beyond (cephalad). Thorax finely scaly, the propodeum glabrous; abdomen delicately scaly. Fore wing with the following remarkable pattern, the venation black except the pale postmarginal vein: A long cone-shaped black marking originating near base in an acute point, running clavately up the center of the blade and a little distad of the apex of the postmarginal vein obliquing up to the cephalic margin just before the apical turn, the obliqued portion narrowing cephalo-distad; a rather narrow apical black stripe (but absent from the cephalo-distal fourth of the apical margin); an oblique (cephalo-proximad), rather narrow stripe from the first long marking at a little before its elbow to the base of the stigmal vein and entirely involving that vein; and another similar stripe but much

shorter, from the first stripe to the base of the marginal vein, the latter pallid except at base. Wings long. Caudal wings dusky at tip, with about a dozen lines of discal cilia. First two tarsal joints long (middle and hind legs). Funicle 1 large, a third longer than wide, 2 subquadrate. Flagellum covered with coarse hairs and stout spicules. Pedicel oval.

From two females collected at San Rafael, Jicoltepec, Mexico.

Type: Catalogue *No. 19376*, U. S. N. M., a female on a tag and a slide with the head, a pair of wings and the middle and hind tibiæ.

There are no bristles on the scutellum. Body naked. Submarginal vein with six long bristles.

2. Genus **Plagiomerus** Crawford.

This genus bears two erect or semi-erect, slender clusters of black hairs at the apex of the scutellum. *Comys cyanea* Ashmead belongs here (identified by P. H. Timberlake) and differs from the genotype in having funicles 3–4 white, the axillæ rather more separated, the scutum apparently more hairy. Yet the two are much alike otherwise. In *Plagiomerus diaspidis* Crawford, the mandibles are quadridentate.

3. **Chalcaspis arizonensis** new species.

Female: Length, 1.50 mm. Short and broad.

Very similar to the genotype, differing notably in that the legs are all metallic green except the tarsi and tips of the middle tibiæ which are reddish like the scape. Antennæ reddish except the bulb of scape and the pedicel which are metallic dark green, the club, however, sometimes darker.

The generic description of *Chalcaspis* is about correct. Head lenticular. Flagellum clavate and with a distinct ring-joint, the funicle joints all much wider than long, 6 largest; club enlarged and as long as the funicle, obliquely truncate from joint 2. Scape long and slender; pedicel somewhat longer than wide. Tegulæ large. Scutum much wider than long. Axillæ separated by a rather long median *suture*. Marginal vein somewhat longer than wide but not against the costal margin (thus in a sense absent). Mandibles strongly *bidentate*. Hind wings short and broad. The body is finely scaly between the punctures. Head wider than the thorax. The fore wing in both species is rather deeply infuscated out to the marginal vein. Compared with type of genotype.

Described from three females on tags in U. S. N. M. (labelled "Santa Rita Mts., Ariz., 10, 6. Hubbard and Schwarz").

Type: Catalogue *No. 19377*, U. S. N. M., one of the above on a tag plus slide with wings and head. Other two females with the same number as paratypes.

4. **Psylledontus secundus** new species.

Female: Length, 0.60 mm.

Differs from the genotype in being smaller, the scutellum is scaly *not* very finely longitudinally striate, funicles 2 and 3 more transverse, also funicle 4; the fore wings are distinctly narrower and the middle tibiæ yellow except a band just below the knee, the caudal tibiæ more broadly yellow at tip. The axillæ are separated by a short carina in both species and the club is obliquely truncate from about half way up one side. Compared with types of the genotype. Face rather strongly inflexed.

Described from four specimens reared at Perideniya, Ceylon (Rutherford) from gall-making psyllids (nymphs).

Type: Catalogue *No. 19378*, U. S. N. M., a female on a tag and a slide bearing the head and fore wings. Three females on tags, same number as paratypes.

5. **Elasmus mexicanus** new species.

Female: Length, 2.60 mm.

Dark metallic blue, the scape, membraneous apex of the postscutellum, tibiæ, tarsi, knees and ends of the femora (all of cephalic femora except at base), yellow. Fore wings hyaline but with a large rounded substigmal spot of fuscous. Hind tibiæ with the dorsal black spines arranged to form a V just under the knee, then two large, long-ovate areas, then an oblique line across near tip. Head very finely scaly and with the usual scattered punctures; pronotum and scutum densely hairy, the scutellum naked (but two or four large bristles may be missing in this specimen), scaly. Propodeum plane, delicately scaly. Abdomen compressed distad, subglabrous, conic-ovate. Middle tibiæ from dorsal or lateral aspect with a narrow marginal stripe of black (each margin) formed by dense black spines. Hind tibial spurs double. Hind coxæ scaly, the femora longitudinally lined, the lines far apart. The long postmarginal vein with fuscous along it. Funicle 1 somewhat over twice longer than wide, 3 somewhat shorter, distinctly longer than the pedicel which is a half longer than wide at apex. Second ring-joint large, the first very short. Mandibles 7-dentate.

Described from one female in the U. S. N. M., collected at San Rafael, Jicoltepec, Mexico.

Type: Catalogue *No. 19379*, U. S. N. M., the specimen on a tag, fore wing and head on a slide.

6. **Elasmus marylandicus** new species.

Female: Length, 2.15 mm.

Dark metallic green-black, the abdomen deep orange yellow, the postscutellum (except at apex) lemon yellow; distal tips of cephalic coxæ and femora and all tibiæ, pale dusky yellow; rest of legs concolorous; black spines on dorsal aspect of caudal tibiæ arranged in three

parallel wavy lines. Tip of valves of the ovipositor, distal fourth of abdomen above, a broad (nearly round) cross-stripe just cephalad of this and cephalad of this (at about the middle of the abdomen), a smaller, semi-circular spot, black. Also cephalad of the latter a mesal dot (at proximal third of the abdomen or less). Venation black, the wings hyaline. Proximal one-eighth of abdomen æneous black. Head with scattered punctures. Scutellum with two pairs of long setæ, otherwise naked, scaly. Funicles 1–3 subequal, each about twice longer than wide, 1 a little more, about twice the length of the pedicel which is subequal to club 3. Mandibles 5- and 6-dentate; two ring-joints.

Described from one female taken by sweeping grass, Chevy Chase Lake, Maryland, April 24, 1915.

Type: Catalogue *No. 19380*, U. S. N. M., the female on a tag, the head on a slide.

7. **Merisus** Walker.

Differs from the pirenine genus *Apirene* Girault in bearing but one spur on the caudal tibiæ and the solid antennal club lacks a distinct terminal nipple, yet is tapering at apex. (The nipple is present sometimes.)

(1) **Merisus flaviventris** new species. Female.

Length, 2.10 mm. Abdomen larger than the rest of the body, depressed. Differs from the genotype of *Apirene* only in that the abdomen is wholly lemon yellow except tip of ovipositor valves (not visible from dorsad) and the brown meson of the abdominal venter. Also the antennal club is white-yellow, the antennæ brown, the scape darker. Base of cephalic femora black. Funicle 1 is shorter in relation to the pedicel, 2 is a little larger than 3, 6 subquadrate, subequal to 3–5. Mandibles 4-dentate.

Described from one female taken by sweeping grass, Chevy Chase Lake, Maryland, April 24, 1915.

Type: Catalogue *No. 19381*, U. S. N. M., a female on a tag, the head and hind legs on a slide.

(2) **Merisus semilongifasciata** new species.

Female: Differs from the preceding species in having the flagellum black except the yellow-white club, the base of the abdomen rather narrowly black and the abdomen with a short, black, lateral marginal stripe (partly broken into three spots) running to about the middle and commencing a short distance away from the basal marginal stripe. Also the funicle joints are all a little longer. Clypeus longitudinally striate.

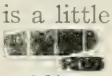
From one female taken with the preceding.

Type: Catalogue *No. 19382*, U. S. N. M., the female and slide as in the preceding.

8. **Aphidencyrtus aspidioti** new species.

Female: Length, 1.45 mm.

Differs most notably from all the species of the genus in having funicles 5-6 white. Dark metallic green, the wings hyaline; tarsi (except distal joint), cephalic and caudal knees, distal half of cephalic tibiæ, base and tips of caudal tibiæ and all of middle tibiæ excepting a rather broad cinctus a rather short distance below the knee, pure white. Funicles 1–4 subequal, distinctly wider than long, 5 and 6 each distinctly larger, 6 subquadrate. Club nearly as long as the funicle and somewhat wider, the middle joint quadrate. Marginal vein 2½ times longer than wide, nearly twice the length of the stigmal, the latter a little longer than the postmarginal. Third tooth of mandible truncate but its distal margin concave, the outer two teeth longer than the inner. Cheeks as long as the eyes. Axillæ barely touching. Thorax scaly. Venation dusky yellow. Hind wings with about twelve lines of discal cilia. Agrees in color with *siphonophoræ* Ashmead except funicles 5 and 6; in the latter funicles 1–4 are subquadrate, the frons is a little broader.



Described from three females reared from *Aspidiotus perniciosus*, Lansing, Michigan, February 9, 1914. Experiment 1001.

Type: Catalogue *No. 19383*, U. S. N. M., a female on a slide.

Aphidencyrtus aphidiphagus (Ashmead) and *A. siphonophoræ* (Ashmead) are the same (types compared). *Encyrtus inquisitor* Howard is a very closely allied species, but the band on the middle tibiæ is much longer and the axillæ are separated. In *A. aphidiphagus*, the male antennæ are 9-jointed, the club solid, the funicle joints clothed with soft hairs, the scape short, compressed; funicle joints cylindrical oval, each about a half the length of the club. There is a pair of the last species in the U. S. N. M., from Washington, D. C. and two males from the same place reared from *Siphonophora liriodendri*, August 22, 1894. The type of *siphonophoræ* Ashmead bears Washington, D. C. as the type locality.

Aphidencyrtus websteri (Howard) differs from *siphonophorae* in having the band on the middle tibiæ as broad as half the length of that joint (female). There are specimens in the U. S. N. M. from Columbus, Ohio and a male reared from *Siphonophora avenæ*.

9. **Coccidencyrtus ensifer** (Howard).

Female: Length, 1.60 mm.

Differs from *Aphidencyrtus aspidioti* in having the marginal vein but slightly longer than wide, somewhat shorter than the stigmal and the postmarginal veins, the funicle and club are pale yellow and there is a small, square fuscous patch against the marginal vein; the funicle joints are all subquadrate, the club is more pointed and nearly equal in length to the funicle. The band on the middle tibia is somewhat longer. The axillæ are slightly separated. The male has the club solid, the funicle and club with rather long, scraggly hairs, the funicle joints cylindrical oval, only somewhat longer than wide, but much shorter than the club.

From several males and females reared from *Aspidiotus juglans-regiæ*, Muskegon, Michigan, July 7, 1914. Received from H. J. Franklin.

EVIDENCE OF A PROTOPLASMIC NETWORK IN THE OENOCYTES OF THE SILKWORM.

By Robert K. Vickery, Stanford University.

In the study of cell morphology the need of discovering some structural framework within the protoplasm has long occupied the mind of the investigators of this field. This subject has received consideration in books published by the late Mr. H. M. Bernard and Dr. Emil Rohde. Independently they have come to the same conclusion, namely, that all tissues and their constituent cells are permeated by a structural network. There is considerable evidence of such a network. In the nucleus the tangle of linin filaments have long been observed. Intracellular filaments have been abundantly demonstrated by a host of workers. Bernard and Rohde have brought out the very significant fact that syncytial tissues are permeated by such networks. The structural character of the cytoplasm has long been a field of controversy. Of the three different early theories—the Fibrillo Reticular Theory, Butschli's Foam Theory, and Altmann's Granular Theory—none has received universal acceptance. The discovery of mitochondria by Benda, Meves, Duesberg and others in all types of cells, has led to considerable speculation as to whether these elements can be truly associated with the linin filaments and their chromatin granules. Also whether they too have any architectural significance. These questions have been largely answered by the work of Michaelis, Chambers and the Lewises by staining the mitochondria of live tissue with Janus Green, and also the cell dissection work of Chambers and Kite. Chambers in his summary, draws the conclusion that the mitochondrial threads have tensile and elastic properties; however, this conclusion is qualified by the statement that these networks are not stable elements but that they reconstruct themselves by chemical or mechanical forces in response to changed conditions. In this paper an attempt will be made to show that in one particular case this network has a definite honeycomb structure and that the mitochondria and the linin filaments are continuous.

The silkworm œnocytes proved to be the most available material. These cells are large ductless glands found in the

body cavity of all immature insects. The arrangement of these glands is in metamerical groups on both sides of the abdomen in conjunction with the spiracles. The cells are usually separate spherical individuals which lie enmeshed or suspended in a tangle of fine tracheæ with which, however, there is no closer connection than that of contact.

The origin of these cells is ectodermal. They arise in the form of a chain by amitotic division from one cell ventro-caudad of each spiracle. From this chain they migrate to their final positions. Before the egg hatches the ultimate number of cells in each group is reached and no further division takes place. These glands persist often into the imaginal stage. The secreting of the cell is a periodic function that takes place even in the embryo. While in the process of secreting the nucleus forms a honeycombed structure and from this long mitochondrial filaments extend to the periphery of the cell. The papers of Holland and Stendell contain good œnocyte reviews and nearly complete bibliographies.

The study of the mitochondria from an architectural point of view has largely been confined to the investigation of the origin of the highly specialized nerve and muscle fibrils. The œnocytes afford an opportunity of studying these structures comparatively free from the specializing influences of mechanical stresses or pressures. The cells originate while the egg is still in a semiplastic state. Immediately on being formed they float away in the body fluid till they come to rest in the delicate meshes of the trachea. For this reason any elemental structure in the cells of the primitive ectoderm might persist through the intervening generations into the make-up of the œnocytes.

With the exception of the eggs the œnocytes are the largest cells to be found in the insect body. This of course has the obvious advantage of making them easy to study. On the other hand when one considers that they are many times the size of the parent ectodermal cells, the question naturally arises as to whether a primitive intracellular network would meet this demand for expansion by the addition of more network or by the stretching of the original. From the observations of the œnocytes it would appear that the latter process is the one that occurs. The network is very open and it is this feature of size that makes it so very distinct.

The direct or amitotic division of these cells brings out certain other interesting conditions. In the writer's preparations the nuclear membrane appears to be totally absent. The very fine granular or colloidal structure of the cell, when brought out by high powers and the dark field illuminator show the nucleus and cytoplasm to have exactly the same physical make-up. The line between the two elements of the cell is shown only by staining. This would indicate that the nucleus and the cytoplasm differ in their chemical make-up rather than in structure as is the case in cells dividing mitotically.

The drawings accompanying this paper will show more clearly than any description the honeycomb structure of the nucleus. Reconstruction of the nucleus in clay makes it even more distinct. The process of formation can be traced in the sections. When the cell is not secreting the nucleus is spherical. It consists at this period of a mass of chromatin granules with which are interspersed about an equal number of drops of the secretion. The drops at this stage are little larger than the chromatin granules. The secreting process begins by the throwing out from the nucleus of several pseudopodia-like processes. These extend in a zigzag way in every direction. The chromatin and the secretion disperse along these lines till at length no large bodies of nuclear material remain. The nucleus has then the appearance of the aforementioned honeycomb. The edges are distinct lines and the vertices coincide in alignment. Around the outer edges of the structure there extend continuations of the edges of the honeycomb. These continuations are usually fine straight or sharply angular lines of particles. They have a striking resemblance in character to the spireme structures of the mitotic nucleus only they invariably follow straight or angular lines.

These long fine filaments respond to the test for mitochondria. They were first found in sectioned material stained with Mayer's alcoholic carmine. This material had been killed and fixed with heat and hardened in alcohol. Some of the same material stained in Hansen's Iron Hæmatoxylin gave clearer results. Benda's modification of the liquid of Flemming as as given by Eklof gave good results with Hansen's Iron Hæmatoxylin and Benda's Alizarin Crystal Violet.

The vital staining of the live cells with Janus Green did not prove to be a very satisfactory method. The cells showed the honeycombed nucleus very clearly but the color reaction for the mitochondrial granules was destroyed by the yellow tint of the cytoplasm. However, the mitochondria could be made out as masses of blue-green granules and filaments. The best strength for the Janus Green (Grubler) proved to be one part in one hundred thousand used in equal amounts with the body fluid.

A feature of these mitochondrial filaments is that they are often grouped around a central vertex. The vertex is analogous to and usually in alignment with the vertices of the honeycomb. It was found possible to measure the angles around these points when both arms lay in the same optical plane. The angular measurements fell into two distinct groups: (1) Angles approaching sixty degrees as a maximum, and (2) a lesser group of angles with an average measure of one hundred and ten degrees. Between four and five hundred actual measurements were taken from about twenty-five slides, from as many different individual larvæ.

Let us consider these data speculatively. From these two angles there can be constructed a hypothetical framework that is ideal from a structural point of view. The unit of such a structure is a tetrahedron. All the angles in one plane in an equilateral tetrahedron are of sixty degrees. The only other angle found in a structure of such units is of one hundred and eight degrees. Any attempt to conjure up a generalized or ideal structural basis of protoplasm is a piece of pure fancy. Yet the angles of the œnocytes fall in with such a plan and the honeycomb structure of the nucleus follows exactly the same lines.

It must be born in mind that the existence of any architectural basis of protoplasm is still a mooted question. This paper is purely speculative to the extent that it is based on the hypothesis that such a structure does exist. The object in taking this for granted is to show by the apparent figures in the œnocytes what would be the simplest character of such a structure. That these peculiar geometrical figures exist is plain to be seen. Whether they have a structural function is open to question. Their geometrical formation might be taken as indirect evidence of such a function. One of the remarkable

points is the continuous character of the linin filaments and the mitochondria. This point of the continuous character of the linin is one of the postulates of the protomitomic theory as expounded by Mr. Bernard.

The work here recorded was done and the paper prepared in the Entomological Laboratory of Stanford University by me as holder of the Bernard Scholarship for 1914–1915 in Insect Histology.

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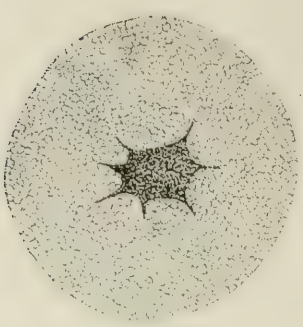
EXPLANATION OF PLATE XXIII.

Fig. 1. Oenocyte in which the process of secreting has just commenced.

Fig. 2. Oenocyte in which the nuclear material is moving out along definite lines.

Fig. 3. Section of an Oenocyte in which the honey-comb structure is appearing and in which only small masses of nuclear material remain.

Figs. 4, 5 and 6. Oenocytes in which sections of the honey-combed nuclei are shown. Note the fine terminal filaments which are mitochondria.



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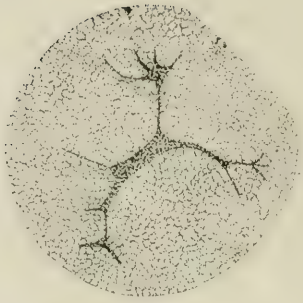

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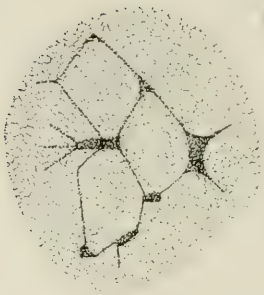

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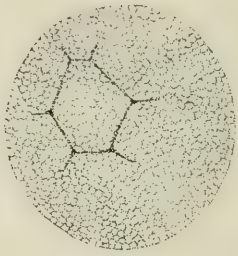

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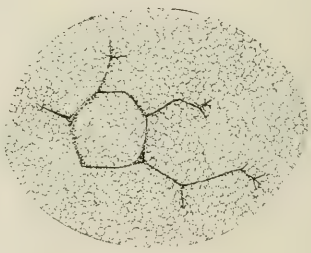

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R. K. Vickery.

ABNORMALITIES AND REGENERATION IN CICINDELA.*

By Victor E. Shelford.

I. Introduction.

The occurrence of certain abnormalities of the elytra and labrum of Cicindela collected in the wild state and in adults reared in the laboratory and the almost entire absence of abnormalities of the legs and antennæ led me to bring together the main facts at my disposal and to perform a few experiments to determine if possible the cause of the abnormalities. It is the purpose of this paper to point out the possibility of the use of the material for studies of the physiology of melanin pigment distribution by operative experiments during development.

II. Types of Abnormalities.

The abnormalities noted are confined chiefly to the elytra and the labrum. Figure 1 shows an abnormal labrum of a specimen of Cicindela tranquebarica of the green Nevada type with the small spots on the margin of the elytron as the only vestige of the usual markings of the species (Wickham '06). This labrum has a slender smooth and shiny projection at one side but suggesting a tearing from the center toward the outside which resulted in the slender strip being left hanging down freely. Such abnormalities are not common.

A second and relatively common type is shown (figures 4 and 5) in which one elytron is longer than the other and the form and color pattern sometimes modified. No abnormal legs have been noted in collected specimens.

III. The Production of Abnormalities in Experiments.

To learn something of the abnormalities of the labrum and legs twenty-six late larvæ and six pupæ of Cicindela punctulata were operated on. The operations were performed with sterile scissors and the larvæ placed in vertical burrows in sterile soil kept moist with a hydrogen peroxide solution. The operations were confined to the labrum and right hind leg.

* Contribution from the Zoological Laboratory of the University of Illinois. No. 46.

Four larvæ were cut in the center of the labrum; six pupæ were operated upon in the same manner. All the pupæ were treated essentially alike being cut from the center of the pupal labrum toward the left side and slightly upward. The results are tabulated below:

Location of cut	Part Removed or Attached	No. Operated on	No. Surviving	Results in Adult
In leg, Tarsal joints	removed	4	2	One showed shorter tarsal joints.
In leg, Tarsal joints	attached	4	3	No modification of adult.
Mid tibia	removed	9	4	No modification of adult.
Mid tibia	attached	2	1	No modification of adult.
Tibia femur joint	removed	3	2	No modification of adult.
Larval labrum in center	attached	4	4	No modification of adult.
Pupal labrum	attached	6	6	4 showed modification 1 recovered completely

The result of removing the legs are all the same except for the one shown in figure 8 which is a little shorter, has a little shorter spur and less numerous and shorter hairs. The pupal legs evidently develop in the upper part of the larval leg and modification results only when the basal portion is injured. Megusar '03, obtained similar results with other beetles. This fact explains the rareness of leg abnormalities.

The experiments on larval labrums gave no results. The pupæ operated on only emerged as adults in three cases. One died before the cuticula hardened. This individual's labrum had completely healed but a very dark area occupied the area near the cut. One that failed to emerge had healed the wound completely. The labrum of the other had almost degenerated. The two seen in figures 2 and 3 show some similarity to the one collected in Nevada. The Nevada specimen suggests that the abnormality is due to a tear in the labrum probably at the time of the last larval moult. The larval cuticula often sticks to the anterior part of the pupa when the surrounding conditions are dry and the Nevada dry climate would favor such accidents.

Elytral abnormalities shown in two species collected in Kansas were duplicated in Cicindela limbalis reared from a larva. Here a reduced color pattern accompanies a short elytron as in the specimen of wild tranquebarica. While this is the only elytral modification of exactly this type noted in wild individuals a type with holes in the elytra is more common in experiments. For example, two holes completely healed occured in an elytron with an irregular and distorted pattern, Fig. 7. While several hundred larvæ of each of several species were reared to maturity this kind of abnormality occurred only three or four times.

The peculiar elytral modifications are probably due to rough handling. It seems probable that the abnormal conditions result from pressure or slight injury during the pupal stage. The elytron being much crowded, the folds projecting outward are particularly liable to injury. The elytral openings in Fig. 7 are rounded and smoothly healed, the wing cavity being entirely closed and with cuticular covering on the edges. The comparatively frequent occurrence of elytral modifications in reared specimens as compared with their rather rare occurrence in nature justifies the above assumption. The abnormalities are not unlike those of Drosophila described by Morgan as mutations. Since the tiger beetle abnormalities occur in animals reared from wild stock which show extremely few such variations in thousands of specimens collected in the wild state an unusual burden of proof is necessary to establish such conditions as anything but abnormalities produced again and again by the necessary rough handling of cultures. If such proved true their apparent inheritance in Drosophila may only be a sensitiveness to handling.

The abnormalities of patterns and the reproduction of black pigment in wounded labral surfaces indicate that such material in the hands of a skillful investigator might, with suitable operations, show something of the physiology of pattern production. If the distribution of pigment can be controlled by suitable operations as is indicated by the work, it will have important bearing on the studies of insect patterns.

University of Illinois, May 19, 1915.

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EXPLANATION OF PLATE XXIV.

Fig. 1. Abnormal labrum of a blue Nevada form of C. tranquebarica.

Figs. 2 and 3. Abnormalities in C. punctata produced by cutting into the labrum during the pupal stage.

Fig. 4. Long and short-elytroned form of C. sexguttata from Topeka, Kansas.

Fig. 5. Specimen of C. tranquebarica with short elytron bearing an abnormal pattern, from Dodge City, Kansas.

Fig. 6. Reared specimen of C. pupurea limbalis a right elytron similar to that of tranquebarica (Fig. 5). Note reduced markings. This deformity is supposedly due to rough handling.

Fig. 7. Elytron of C. limbalis with healed openings due to the death of certain parts of the folded pupal elytron.

Fig. 8. Right short hind leg of a specimen of C. punctulata in which the tarsal joints were removed just before pupation. The hairs are not well developed.

Fig. 9. Left normal hind leg of the same specimen of C. punctulata.

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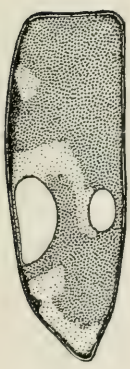

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V. E. Shelford.

INTERESTING WESTERN ODONATA.

By Clarence Hamilton Kennedy,
Stanford University, California.

In the following notes I wish to give a short account of the habits of some of the more interesting species of western Odonata. These are based on field observations made by the writer in Washington, Oregon, California and Nevada during the summers of 1913 and 1914.

Apparently because of the actual scarcity of streams in the west, various species of Odonata in their attempts to utilize all available water have taken on unusual habits or have developed more ordinary habits in some special direction to such a degree that they have almost assumed the grotesque in their exaggeration. One of these which might be said to have exaggerated habits is *Archilestes californica*. This species, known heretofore from the type and a single other specimen, is abundant in the Yakima Valley, Wash., and throughout Central California. It is a giant Lestes, differing from the numerous species of that cosmopolitan genus in greater size and minor venational characters.

The species of Lestes oviposit endophytically and frequently a foot or even two feet above the surface of the water, usually placing the eggs in such tender tissues as the stems of sedges and Juncus, or occasionally in tender willow shoots. In oviposition Archilestes follows the habits of its Lestes relatives, but because of its greater size and strength it oviposits normally from five to eight feet above the water and in the bark of willow stems that are from a half an inch to an inch and a half in diameter. Because of the size of some of the bushes used, it can almost be said to be a dragon fly that lays its eggs in trees. Oviposition is a tedious process. The male holds the female by attaching the claspers on the end of his abdomen to the posterior edge of her prothorax. Then with her abdomen bent in a loop she forces her ovipositor slowly thru the bark and deposits her eggs in clutches of six in the cambium, where they remain dormant until the following spring. The hatching has not been observed, altho it is probable that the larvae wriggle from the bark and fall into the water below. The circle of bark, under

which lies each clutch of eggs, dies after the eggs have hatched and this produces a scar, which from year to year increases laterally with the growth of the stem. Sometimes the scars of contiguous ovipositions run together and girdle a willow, so this dragonfly may be classed technically among those insects injurious to timber.

The nymphs of this dragonfly, which are probably among the largest of the Zygopterus nymphs, are peculiar in being very free swimming. On the Yakima River I worked for an hour with a rake without catching a single specimen, tho I knew from their emergence that they must be abundant. Later in California I was astonished to discover that certain "schools of minnows" were the agile nymphs of Archilestes fleeing enmasse from the dragonfly collector. On closer observation they were found to spend most of their time resting quietly on submerged objects, but on the approach of danger they fled precipitously to deeper water. Swimming was accomplished by a vigorous undulatory motion, in which the large caudal gills seemed of great assistance.

One of the peculiarities of the western Odonate fauna is the small number of Argias. This is a genus of 60 or more species the greater number of which are found in the American tropics. Eight or more occur in the eastern states, but only two are found west of the Rockies, excepting, of course, the various southern species limited to the Mexican border. Tho essentially a tropical genus, one of the two western species, *Argia emma*, is common as far north as central Washington, and the other *Argia vivida*, is found even as far north as Canada. The extraordinary northern distribution of *Argia vivida*, which occurs also as far south as southern Mexico, seems explained by its peculiar habits. All the species of *Argia* as far as is known, live in very fresh water, the majority of them being stream species. Such is *Argia emma*, which is found in the majority of the warm perennial streams of the west. But *Argia vivida* has a special preference for springs and the boggy streamlets flowing from them. This species is frequently collected on larger streams and ponds, but in such cases, when traced to its origin, is found to be emerging from some nearby spring. Now springs do not freeze, as their waters, originating deep in the ground, maintain a fairly uniform temperature

thruout the year, so that the springs in western Canada, in which *Argia vivida* has been taken are probably the warmest waters in that region, comparing not unfavorably in warmth with springs of California and even Mexico. Thus by inhabiting springs this little subtropical Argia easily maintains itself far beyond the usual limits of members of its genus. Its vertical distribution is equally as great as it occurs from sea level up to 6,000 feet, where it is found in springs on the shores of Lakes Tahoe and Donner.

An interesting observation in this connection is that *Argia emma*, the warm stream species, occurs also at this altitude, being found in the Truckee River at the outlet of Lake Tahoe. This is explained by the fact that the Truckee River, tho a mountain torrent and at an elevation of 6,000 feet is really a warm stream, because its supply comes from Lake Tahoe, which never freezes over. The great depths of this lake are filled during the summer with a body of water of 39° or more in temperature. (Actual measurements in August show 40° or more except in extreme depths). Tho the surface chills during the cold season, the lake waters are constantly turned over by the winter winds, bringing the warmer waters to the surface, where they keep the Truckee River supplied thruout the cold months with water several degrees above freezing.

Another Agrionine with unusual habits is *Enallagma clausum*. This is an inhabitant of the desert and seemingly enjoys its life in the alkaline ponds of this barren region. It is found in the intermountain country from the Columbia Valley to Nevada. Several species of this large genus are stagnant water species and some of these in the West live in ponds with a slight alkaline content, but *clausum* goes beyond them all and breeds in water strongly saline, for it is found breeding in large numbers in the shallow edges of Pyramid Lake, Nevada. This is one of those salt lakes in the midst of the Nevada desert which have been left by the gradual drying up of Lake Lehontin. While the alkalinity of Pyramid Lake water is but about one tenth of that of sea water, it is very near the maximum that can be endured by various brackish water species. Sea water has a density of 1.026. Osburn (Am. Nat. June, 1906), has shown that various species of odonate nymphs found commonly in brackish coast ponds can endure a density of not more than

1.003. Pyramid Lake water has a density of 1.00347 to 1.00349. The broad sandy beach at the southern end of Pyramid Lake fairly swarms with the nervous imagoes of this species, and the females accompanied by the males oviposit in the masses of filamentous algae that float in the shallow edge of the water.

Oddly enough *Enallagma clausum* shares its occupancy of Pyramid Lake with another dragon fly, a libelluline, *Sympetrum corruptum*, which just opposite to *Enallagma clausum*, instead of being a species restricted by special habits, is extremely adaptable, in fact the dragonfly, which in the west, is found in a greater variety of environments than any other. *Sympetrum corruptum* occurs from the sea level to altitudes of 3,500 feet in Washington and 5,000 feet in California, and flourishes not only in all kinds of ponds, but in all streams except those very swift mountain torrents inhabited by Octogomphus and Cordulegaster. Thus it is interesting that the extreme environmental condition found in the salinity of Pyramid Lake has been mastered as it were thru opposite types of development; by extreme specialization in *clausum* for a life in alkaline water, and by an extreme generalization in *Sympetrum corruptiun* for a life in the greatest variety of waters. Both species flourish side by side and no other species was observed.

Many interesting phases of odonate habits and distribution in the West are related in various ways to the very rugged topography of this region. In a half day one can go by train from sea level in the Sacramento Valley, with its Mexican fauna and sprinkling of tropical species, to an elevation of 6,000 feet in the Sierras, where all odonate species are such as are found in Canada. These northern species are in various ways adapted to endure the cold, which prevents the occupation of these high altitudes by multitudes of dragonflies that flourish in the sunshine of the warm valleys. A special adaption, which permits one of these species to exist on this cold upper limit of odonate life was discovered while collecting about the McKinney Lakes, which lie on the divide west of Lake Tahoe, at an elevation of 7,000 feet. The species in question is the large blue *Aeshna interrupta nevadensis*. This is restricted to the summits of the Sierras having been found from an elevation of 4,000 feet at Emigrant Gap to 7,000 feet on the McKin-

ney Lakes. Five other species of Aeshna are found in California. These are scattered from the sea level up to 5,000 feet altitude, but *nevadensis* is the only form which flourishes at the extreme upper limit of 7,000 feet. The adaption, which permits this one species to occupy territory so far beyond the range of the genus in general, is a change in the time of emergence. As far as is known the species of Aeshna emerge in the nighttime, an adaption to preserve them from the birds, but *nevadensis* emerges in the day time. At this high altitude there are few species of birds that inhabit the shores of the lakes, so night emergence is not necessary. Day emergence, however, is a necessity as the nightly temperature at this altitude is very near freezing, if not actually at times below. It is interesting to note here that night emergence in Aeshna is a highly specialized habit, as most Odonata emerge in the day time, and that *nevadensis* belongs to one of the more generalized or primitive groups of the genus. Perhaps it has merely retained the primitive manner of emergence.

For two western species an interesting form of migration was observed. These are *Cordulegaster dorsalis* and *Octogomphus specularis*. The coast mountains of California and the western slope of the Sierras contain many perennial torrents, which do not rise high enough to contain snow water, yet occur in such steep gulches that they are a succession of rushing rapids and roaring cascades. These are inhabited by but three species of dragonflies, Cordulegaster, Octogomphus and an undescribed species of Aeshna. The nymphs of this Aeshna are agile, active creatures entirely able to stem the swift currents of these torrents, but the nymphs of Cordulegaster and Octogomphus are slow and clumsy. Moreover they do not live in the tree roots as do the Aeshna nymphs, but in the case of Cordulegaster, crawl over the bottom in the quieter parts of the pools, and, in the case of Octogomphus, burrow thru the organic trash in the deeper holes. Being, as it were, loose in the stream these two, during their three-year life, are washed farther and farther down stream by each succeeding freshet, so that when they come to emerge they may find themselves several miles below the point at which they hatched. This washing down is compensated by a migration upstream of the imagoes. On Stevens Creek, south of Stanford Uni-

versity, where these were most fully observed, exuviae were found in abundance two miles below the lowest point on the creek at which any imagoes were seen, and imagoes were common on the divide at the head of the creek, where few exuviae were found. These observations were checked in other parts of California. It is this migration upstream which keeps Cordulegaster and Octogomphus limited in their distribution to the head waters of these torrents and prevents their even occasional appearance in the lower level reaches of these same streams.

Cordulegaster is one of those strange insects with unusual structure and equally unusual habits, and as it lives on the headwaters of the wildest mountain streams, but little has been known concerning it. The ovipositor of the female is very long and heavy, trough-shaped affair and very blunt. After many conjectures as to how it was used, Dr. Ris finally succeeded in observing the female of a Swiss species in the act of ovipositing. This for a dragonfly was a very unusual operation. Most Zygoptera oviposit by inserting eggs in vegetable tissues with the aid of their needle-like ovipositors. Most Anisoptera oviposit by washing the eggs into the water from the tip of the abdomen. Cordulegaster does neither. The female observed flew hastily up the creek examining each sandy shallow, until she finally found one protected on three sides by stones in the water, and not over an inch deep. She hovered over this and dropping her long abdomen into a vertical position made a series of dips or backward plunges, at each dip thrusting the tip of the abdomen with its heavy blunt ovipositor thru the shallow water into the sand beneath. After perhaps a half dozen thrusts she flew up the creek a short distance and finding another shallow to her liking repeated the process. The whole process rather closely resembled the manner of oviposition of some of the crane flies.

In the canyon back of Mt. Lowe at Pasadena, Cal., I had an opportunity of observing the habits of the tropical *Paltothemis lineatipes*. This is a large red-bodied libelluline with habits of flight, which in part resemble those of a corduline and in part the high flying habits of a Tramea or Pantala. The nymphs are as interesting as the adults and were very abundant in the clear mountain stream. This flows thru a gorge whose

rocks are a coarse granite, which readily disintegrates into a very coarse sand, that is creamy white with numerous dark brown and black grains. The bed of the stream, where shallow, is composed of this coarse sand, giving it a peppered or even checkered appearance. In the deeper and swifter channels this sand is displaced by gravel and rocks. The nymphs of Palto-themis apparently go thru from two to three years of nymphal life before emerging. For the first two years the young nymphs crawl about over the coarse spotted sand of the shallows, but in the last year they live altogether in the deeper water. During the early stages while the young are living on the spotted grit bottom they have a very striking black and white checkered coloration, which lets them blend wonderfully well into their background of checkered sand; but in the later stages when they are on an ordinary bottom in the deeper channels they have the usual olive brown of most large odonate nymphs.

CORRECTION.

(**Athysanus villicus** Crumb=) **Deltocephalus colonus** Uhler

Too late to recall the description, I learn that my *Athysanus villicus* is a synonym of *Deltocephalus colonus* Uhler. This species was described from the island of St. Vincent.

S. E. Crumb.

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HERBERT OSBORN, Managing Editor,
ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA,
State University, Columbus, Ohio.

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